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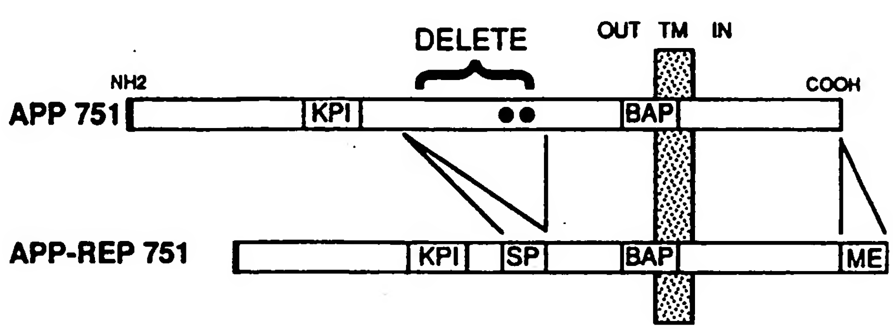
(54) Novel amyloid precursor proteins and methods of using same.

(57) This invention provides novel nucleic acid molecules which encode amyloid precursor muteins and the polypeptides encoded therefrom. Also provided are host vector systems useful for the recombinant production of the recombinant polypeptides in procaryotic and eucaryotic systems. Cells comprising the host vector systems of this invention as well as methods of recombinantly producing these polypeptides are provided by this invention. Further provided is a method to detect the recombinant polypeptides of this invention.

Figure 1.



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BACKGROUND OF THE INVENTION

Throughout this application various references are referred to within parentheses. Disclosur s of these publications in their entirety are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citations for these references may be found at the end of this application, immediately preceding the claims.

Abnormal accumulation of extracellular amyloid in plagues and cerebrovascular deposits are characteristic in the brains of individuals suffering from Alzheimer's disease (AD) and Down's Syndrome (Glenner and Wong, BBRC, 120:885-890, 1984; Glenner & Wong, BBRC, 120:1131-1153, 1984). The amyloid deposited in these lesions, referred to as beta amyloid peptide (BAP), is a poorly soluble, self-aggregating, 39-42 amino acid (aa) protein which is derived via proteolytic cleavage from a larger amyloid precursor protein (APP) (Kang et al., Nature 325:733-736, 1987) BAP also is thought to be neurotoxic (Yankner et al.,Science 245:417-420, 1990). APP is expressed as an integral transmembrane protein (Dyrks et al., EMBO. J., 7:949-957, 1989) and is normally proteolytically cleaved by "secretase" (Sisodia et al., Science, 248:492-495, 1990; Esch et al., Science, 248:1122-1124) between BAP-16K (lysine) and - 17L (leucine). Cleavage at this site therefore precludes amyloidogenesis (Palmert et al., BBRC, 156:432-437, 1988) and results in release of the amino-terminal APP fragment which is secreted into tissue culture medium (Sisodia et al., ibid, Esch, et al., ibid). Three major isoforms of APP (APP-695, APP-751 and APP-770 amino acids) are derived by alternative splicing (Ponte, et al., Nature 331:525-527, 1988; Kitaguchi et al., Nature 331:530-532, 1988; and Tanzi, et al., Nature 331:528-530, 1988), are expressed as integral transmembrane proteins (Kang et al., Nature 325:733-736, 1987; Dyrks et al., EMBO J. 7:949-957, 1988).

Even though both APP-770 and -751 isoforms contain a protease inhibitor domain, it is the secreted portion of APP-751 (also known as Protease Nexin II (Van Nostrand et al., Science, 248:745-748, 1990) which is thought to be involved in cell adhesion (Schubert et al., Neuron, 3:689-694, 1989), remodeling during development, coagulation (Smith et al., Science, 248:1126-1128, 1990) and wound repair.

Although the mechanisms underlying abnormal proteolytic processes which result in BAP extraction from APP are poorly understood, it is thought to be central to the pathogenesis (Selkoe, Neuron, 6:487-498, 1991; Isiura, J. Neurochem. 56:363-369, 1991) and memory loss (Flood, et al., Proc. Natl. Acad. Sci. 88:3363-3366, 1991) associated with Alzheimer's Disease.

Based on the observations that (a) amyloid plagues develop in AD brains, (b) a major component of plagues is BAP, (c) BAP is generated by proteolytic cleavage of APP protein, (d) mRNA levels of specific APP isoforms increase in AD suggesting that more APP protein is expressed, (e) APP point mutations which are thought to possibly after normal processing have been identified in Familial AD (FAD) and "Dutch" disease, (f) injection of BAP into the brains of rodents both form lesions reminiscent of plague pathology and result in memory deficits, and (g) the detection of plague-like amyloid deposits in the brains of transgenic mice expressing human APP, it is important to understand how APP is processed to generate BAP.

SUMMARY OF THE INVENTION

This invention provides novel nucleic acid molecules which encode amyloid precursor muteins and the polypeptides encoded therefrom. Also provided are host vector systems useful for the recombinant production of the recombinant polypeptides in procaryotic and eucaryotic systems. Cells comprising the host vector systems of this invention as well as methods of recombinantly producing these polypeptides are provided by this invention. Further provided is a method to detect the recombinant polypeptides of this invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Schematic representation of APP-REP 751. APP-REP 751 represents a cleavable APP substrate system which contains target sequences of BAP including normal flanking regions (not to scale). The APP-REP protein is marked with a 276 amino acid deletion (corresponding to APP-751 beginning at XhoI through to and including the glycine codon at 15 amino acid residues N-terminal to BAP) and the insertion of sequences encoding N- and C- terminal reporter epitopes. Substrate P (SP) reporter epitope (RPKPQQFFGLM) is inserted at the XhoI site. Met-enkephaline (ME) reporter epitope (YGGFM) is inserted at the C-terminus of APP. The resulting construct encodes 492 amino acids (see Figure 2).

Figure 2: Schematic representation depicting the construction of APP-REP from APP-751 cDNA. Partial representing N- and C-terminal regions of APP-REP were cloned separately as illustrated below. The N-

terminal partial was constructed by ligating sequences encoding substance P (SP) to an N-terminal fragment of APP cDNA. The C-terminal partial was constructed by PCR amplification using the corresponding portion of APP cDNA to introduce novel ends including the Met-enkephalin (ME) r porter epitope. A functional APP-REP 751 clone was obtained by subcloning the partials as indicated. EcoRI (E), XhoI (X), HindIII (H), BamHI (B), SalI (S), XbaI (Xb).

Figure 3: Epitope mapping of APP-REP 751 expressed in COS-1 cells. Immunoprecipitation analysis of cell lysate and conditioned medium using the SP (anti-N-terminal substance P reporter) and M3 (anti-C-terminal APP) antisera. Lanes 1 and 2, cell lysate immunoprecipitated with SP and M3 antisera, respectively; lanes 3 and 4, conditioned medium immunoprecipitated with M3 and SP antisera, respectively; lanes 5 and 6, conditioned medium of control cells transfected with vector DNA immunoprecipitated with SP and M3 antisera, respectively; lane M, molecular weight markers.

Figure 4: Pulse-chase analysis of APP-REP 751. Immunoprecipitation of cell lysate (A) and CM (B). COS-1 cells were pulsed with [^{35}S]-methionine for 15 minutes and chased using cold methionine for 0, 0.5, 1, 1.5, 2 and 4 hours (lanes 1 to 6). Lanes 7, 8 and 9 are chase intervals of 0, 1 and 2 hour for control cells transfected with vector DNA. Lane M, molecular weight markers.

Figure 5: Epitope mapping and comparative expression of APP-REP 751, $BAP_{E22Q}$and $BAP_{\Delta}{}^{11-28}$.A, Schematic representation of relevant BAP (boxed) and flanking amino acid sequences of APP-REP 751, $BAP_{E22Q}$and $BAP_{\Delta 11-28}$juxtaposed against the putative transmembrane domain (shadowed). B-F, Immunoprecipitation analysis with antibodies recognizing indicated substance P (SP), KPI domain (KPI), C-terminal APP (M3) or Met-enkephalin (ME) epitopes; Lane M, molecular weight marker. B, Conditioned medium obtained from COS-1 cells expressing APP-REP 751 (lane 3), BAP_{E22Q}(lanes 4, 6 and 8), $BAP_{\Delta 11-28}$(lanes 5, 7 and 9) or control cells with (lane 2) or without (lane 1) transfection with vector DNA. C, Cell lysates obtained from COS-1 cells expressing APP-REP BAP_{E22Q}(lanes 1, 4 and 7), $BAP_{\Delta\ 11-22}$ (lanes 2, 5 and 8) and control cells transfected with vector DNA (lanes 3, 6 and 9). D, Accumulation of secreted APP-REP 751 fragments in the conditioned medium obtained from COS-1 cells expressing APP-REP 751 (lanes 2 and 6), BAP_{E22Q} (lanes 3 and 8), $BAP_{\Delta 11-28}$(lanes 4 and 7), or control cells transfected with vector DNA (lanes 1 and 5), were pulsed with [^{35}S]-methionineand chased for 45 (lanes 1-4) or 90 (lanes 5-8) minutes with cold methionine. E, Accumulation of secreted APP-REP fragments in the conditioned medium obtained from stable (Chinese hamster ovary cells; lanes 1-4) and transient (COS-1 cells; lanes 5 and 6) expression of APP-REP 751 (lanes 2 and 5), $BAP_{\Delta 11-28}$ (lanes 3 and 6), BAP_{E22Q} (lane 4), or control cells transfected with vector DNA (lane 1).

Figure 6: Peptide mapping and sequencing of fragments secreted into the conditioned medium obtained from Chinese hamster ovary cells stably expressing APP-REP 751, BAP_{E22Q} and $BAP_{\Delta 11-28}$. **A**, Schematic representation depicting the APP-REP 751 and related derivative indicating the cleavage products and relevant carboxy-terminal fragments derived from treating the secreted fragments either with BNPS-Skatole (**B**) or cyanogen bromide. Downward- or upward-facing arrows represent BNPS-Skatole and cyanogen bromide cleavage sites, respectively. Amino acid lengths of relevant fragments for mapping or sequencing are given. **B**, BNPS-Skatole treatment of fragments secreted into the conditioned medium obtained from CHO cells stably expressing APP-REP 751 or $BAP_{\Delta 11-28}$. Mixture of conditioned medium containing APP-REP and $BAP_{\Delta 11-28}$(lane 1), or $BAP_{\Delta 11-28}$(lane 2) and APP-REP 751 (lane 3) alone.

Figure 7: Nucleotide and amino acid sequence of the APP-REP 751 protein.

Figure 8: Nucleotide and amino acid sequence of APP 770 which also is available from the Genebank data base under accession number Y00264.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a nucleic acid molecule encoding an amyloid precursor mutein, wherein the nucleic acid molecule comprises, from the 5' end to the 3' end, a nucleic acid sequence encoding a marker and a nucleic acid sequence encoding the amino terminus of APP up to but not including the sequences that encode BAP. These nucleic acid molecules may include, but are not limited to the nucleic acid molecules selected from the group consisting of pCLL983, pCLL935, pCLL934 and pCLL913.

This invention also provides a nucleic acid molecule encoding an amyloid precursor mutein, wherein the nucl ic acid molecul compris s, from the 5' nd to the 3' end a nucleic acid s quenc encoding BAP and a nucleic acid sequence encoding a marker. These nucleic acid molecules may include, but are not limited to the nucleic acid molecules selected from the group consisting of pCLL947, pCLL914, pCLL937, pCLL949 and pCLL957.

Further provided by this invention is a nucleic acid molecul which comprises the nucleic acid molecul s defined her inabove to ach other. Method of ligating are well known to thos of skill in the art.

These nucleic acid molecules may include, but are not limited to the nucleic acid molecules selected from the group consisting of pCLL618, pCLL619, pCLL620, pCLL600, pCLL964, pCLL962, pCLL989, pCLL987, pCLL990, pCLL988, pCLL601, pCLL602, pCLL603, pCLL604, pCLL605, pCLL606 and pCLL607.

As used herein, the term "amyloid precursor mutein" is intended to encompass an amyloid precursor protein that is mutated, i.e., it is derived from a nucleic acid molecule which has changes in its primary structure as compared to wild-type amyloid precursor protein (APP). Wild-type APP exists in three isoforms, thus, the nucleic acid molecule is changed in its primary structure for each of the three isoforms of wild-type APP. As is known to those of skill in the art, a mutation may be a substitution, deletion, or insertion of at least one nucleotide along the primary structure of the molecule. The mutations which are encompassed by this invention are the result of saturation mutagenesis in the regions of APP which are susceptible to cleavage by endoproteolytic enzymes. These mutations include deletions of nucleic acids encoding particular amino acids, substitution of nucleic acid sequences encoding one amino acid for a different amino acid and addition of nucleic acid sequences encoding additional amino acids not present in the wild type APP sequence. The term "marker" encompasses any substance capable of being detected or allowing the nucleic acid or polypeptide of this invention to be detected. Examples of markers are detectable proteins, such as enzymes or enzyme substrates and epitopes not naturally occurring in wild-type APP that are capable of forming a complex with an antibody, e.g. a polyclonal or monoclonal antibody. In the preferred embodiment of this invention, the marker is an epitope that is capable of being detected by a commercially available antibody. In one embodiment, the marker is an epitope capable of being detected by a monoclonal antibody directed to the Substance P, the Met-enkephalin or the c-myc epitope. In the most preferred embodiment of this invention, the marker is the c-myc epitopic region.

The term "BAP region" is defined as the region of APP wherein endoproteolytic cleavage will yield the amino-terminus and the carboxy-terminus of the BAP which is deposited as plagues and cerebrovascular amyloid in Alzheimer's disease brain. The function of the "BAP region" is to give rise to BAP which may function as a neurotoxic and/or neurotrophic agent in the brain and as other functionalities ascribed to BAP. The "BAP region" may also be endoproteolytically cleaved by enzymes. Such enzymes may include, but are not limited to the enzymes multicatalytic prtenase, propyl-endopeptidase, Cathepsin-B, Cathepsin-D, Cathepsin-L, Cathepsin-G or secretase. Secretase cleaves between lysine-16 (K-16) and leucine-17 (L-17) where full length BAP comprises the amino acid sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNK-GAIIGLMVGGVVIA. Thus, for the purposes of this invention, the preferred embodiment is a cDNA which encodes an RNA which is translated into a protein which is the substrate for endoproteolytic activities which generate BAP.

In addition, for the purposes of this invention, the nucleic acid molecule may be DNA, cDNA or RNA. However, in the most preferred embodiment of this invention, the nucleic acid is a cDNA molecule.

This invention also encompasses each of the nucleic acid molecules described hereinabove inserted into a vector so that the nucleic acid molecule may be expressed, i.e., transcribed (when the molecule is DNA) and translated into a polypeptide in both procaryotic and eucaryotic expression systems. Suitable expression vectors useful for the practice of this invention include pSVL (Pharmacia), pRCRSV (Invitrogen), pBluescript SK$^+$ (Stratagene), pSL301 (Invitrogen), pUC19 (New England Biolabs). However, in the preferred embodiment of this invention, the vector pcDNA-1-neo is the expression vector for expression in eucaryotic cells. As is well known to those of skill in the art, the nucleic acid molecules of this invention may be operatively linked to a promoter of RNA transcription, as well as other regulatory sequences. As used herein, the term "operatively linked" means positioned in such a manner that the promoter will direct the transcription of RNA off of the nucleic acid molecule, An example of a promoter is the human cytomegalovirus promoter. The vectors of this invention preferably are capable of transcribing and/or translating nucleic acid in vitro or in vivo. The recombinant polypeptides produced from the expression of the nucleic acid molecules of this invention are also provided.

A host vector system for the production of the recombinant polypeptides described hereinabove and for expressing the nucleic acid molecules of the subject invention are provided. The host vector system comprises one of the vectors described hereinabove in a suitable host. For the purpose of the invention, a suitable host may include, but is not limited to a eucaryotic cell, e.g., a mammalian cell, a yeast cell or an insect cell for baculovirus expression. Suitable mammalian cells may comprise, but are not limited to Chinese hamster ovary cells (CHO cells), African green monkey kidney COS-1 cells, and ATCC HTB14 (American Type Tissue Culture). Most preferably, the cell lines CRL 1650 and CRL 1793 are us d. Each of these are available from the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland U.S.A. 20852. Suitable procaryotic cell may include, but are not limited to bacteria c lls, HB101 (Invitrogen), MC1061/P3 (Invitrogen), CJ236 (Invitrogen) and JM109 (Invitrogen). Accordingly, the procaryotic or eucaryotic cell comprising the vector system of this invention is further provided by this

invention.

As is known to those of skill in the art, recombinant DNA technology involves insertion of specific DNA sequences into a DNA vehicle (vector) to form a recombinant DNA molecule which is capable of being replicated in a host cell. Generally, but not necessarily, the inserted DNA sequence is foreign to the recipient DNA vehicle, i.e., the inserted DNA sequence and DNA vector are derived from organisms which do not exchange genetic information in nature, or the inserted DNA sequence comprises information which may be wholly or partially artificial. Several general methods have been developed which enable construction of recombinant DNA molecules. For example, U.S. Patent No. 4,237,224 to Cohen and Boyer describes production of such recombinant plasmids using processes of cleavage of DNA with restriction enzymes and joining the DNA pieces by known method of ligation.

These recombinant plasmids are then introduced by means of transformation or transfection and replicated in unicellular cultures including procaryotic organisms and eucaryotic organisms and eucaryotic cells grown in tissue culture. Because of the general applicability of the techniques described therein, U.S. Patent No. 4,237,224 is hereby incorporated by reference into the present specification. Another method for introducing recombinant DNA molecules into unicellular organisms is described by Collins and Hohn in U.S. Patent No. 4,304,863 which is also incorporated herein by reference. This method utilized a packaging, transduction system with bacteriophage vectors (cosmids).

Nucleic acid sequences may also be inserted into viruses, for example, a vaccinia virus or a baculovirus. Such recombinant viruses may be generate, for example, by transfection of plasmids into cells infected with virus, Chakrabarti et al, (1985) Mol. Cell Biol. 5:3402-3409.

Regardless of the method used for construction, the recombinant DNA molecule is preferable compatible with the host cell, i.e., capable of being replicated in the host cell either as part of the host chromosomes or as an extrachromosomal element. The recombinant DNA molecule or recombinant virus preferable has a marker function which allows the selection of the desired recombinant DNA molecule(s) or virus, e.g., baculovirus. In addition, if all of the proper replication, transcription and translation signals are correctly arranged on the recombinant DNA molecule, the foreign gene will be properly expressed in the transformed or transfected host cells.

Different genetic signals and processing events control gene expression at different levels. For instance, DNA transcription is one level, and messenger RNA (mRNA) translation is another. Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes RNA synthesis. The DNA sequences of eucaryotic promoter differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno (SD) sequence on the mRNA. For a review on maximizing gene expression, see Roberts and Lauer (1979) Methods in Enzymology 68:473.

Many other factors complicate the expression of foreign genes in procaryotes even after the proper signals are inserted and appropriately positioned. One such factor is the presence of an active proteolytic system in E. coli and other bacteria. This protein-degrading system appears to destroy foreign proteins selectively. A tremendous utility, therefore, would be afforded by the development of a means to protect eucaryotic proteins expressed in bacteria from proteolytic degradation. One strategy is to construct hybrid genes in which the foreign sequence is ligated in phase (i.e., in the correct reading frame) with a procaryotic structural gene.

Expression of this hybrid gene results in a recombinant protein product (a protein that is a hybrid of procaryotic and foreign amino acid sequences).

Successful expression of a cloned gene requires efficient transcription of DNA, translation of the mRNA and in some instances post-translation modification of the protein. Expression vectors have been developed to increase protein production from the cloned gene. In expression vectors, the cloned gene is often placed next to a strong promoter which is controllable so that transcription can be turned on when necessary. Cells can be grown to a high density and then the promoter can be induced to increase the number of transcripts. These, if efficiency translated, will result in high yields of polypeptide. This is an especially valuable system if the foreign protein is deleterious to the host c ll.

Several r combinant DNA expression systems are describ d below in th Experimental Procedures section for the purpose of illustration only, and these examples should not be constru d to limit the scope of the present invention.

A method for producing a recombinant polyp ptid described hereinabove, is also provided. This method comprises growing the host cell containing the nucleic acid of this invention and/or the host vector

system of this invention under suitable conditions, permitting production of the polypeptide and recovering the resulting recombinant polypeptide produced.

A method of detecting in a sample the pr sence of any of the recombinant polypeptides described hereinabove is further provided by this invention. In the preferred embodiment of this invention, the marker is an epitope directed against an antibody, the epitope of which is not present in the wild-type polypeptide or APP derivative. This method comprises obtaining a sample suspected of containing the polypeptide and contacting the sample with an antibody directed to the marker. The contacting is done under suitable conditions to favor the formation of an antibody-epitope (i.e., antigen) complex, and detecting the presence of any complex so formed. The presence of complex being a positive indication that the recombinant polypeptide is in the sample. In one embodiment of this invention, the antibody is a mouse antibody. In another embodiment of this invention, the antibody is a human antibody. In the most preferred embodiment, the mouse or human antibody is either a mouse or human monoclonal antibody.

The antibody is labeled with a detectable marker selected from the group consisting of radioisotopes, dyes, enzymes and biotin. For the purposes of this invention, suitable radioisotopes include, but are not limited to, ^{32}P, ^{35}S, ^{131}I and ^{125}I.

Suitable samples for the practice of this invention include, but are not limited to conditioned mediua, cell lysates and cellular organelle fractions.

The method of this invention may utilize the recombinant polypeptide for the detection of drugs or compounds that inhibit or augment the activity of proteolytic enzymes which cleave APP to generate BAP fragments. For the purposes of example only, a recombinant polypeptide which contains a Substance-P marker epitope on the amino-terminal side of BAP and a Met-enkephalin marker epitope on the carboxy-terminal side of BAP. Using commercially available RIA kits (Peninsula), one can measure the amount of amino-marker and carboxy-marker in any given sample. Since endoproteolytic activity is shown (see Figure 3) to allow the release of amino-terminal fragments of APP containing the amino-marker into the conditioned media while carboxy-terminal APP fragments containing the carboxy-marker remain associated with the cell, then RIA which measure the amount of amino-marker in the conditioned medium as a direct result of endoproteolytic cleavage activity between the marker epitopes preferable within the "BAP region". Using this RIA to the amino-marker, the effect of potential drugs designed to modify endoprotease activity can be tested comparing the level of amino-marker in untreated and endoprotease-inhibitor treated samples. If a difference in non-treated and treated samples is found, then the position of the cleavage or lack of cleavage can be verified as with the procedures used in Figures 3 to 6. Thus, the qualitative and quantitative aspects of endoproteolytic activity and its inhibition on the recombinant APP mutein is evaluated. The amino-marker also is an enzyme such as betagalactosidase which would be released int the conditioned media by the action of an appropriate endoprotease. Cell free samples of conditioned media containing the liberated enzyme converts a chromogenic substrate into the appropriately colored product (Blue for X-gal and Yellow for ONPG) which is measured spectrophotometricallY. Inhibitors of the appropriate endoprotease would inhibit the release of betagalactosidase enzyme into the conditioned medium resulting in less colored product being observed.

It is a purpose of this invention to develop a cleavable APP substrate system which represents target sequences of BAP including normal flanking regions to provide recognition sequences for processing enzymes. The utilization of a common substrate for parallel strategies involving in vitro cleavage assays using cellular extracts in vivo processing assays in tissue culture or bacterial cells, or in conjunction with a selection system aimed at cloning BAP-cleaving proteases (or other relevant proteins) is preferred.

A second purpose of this invention is to develop an APP substrate which is non-cleavable by secretase in order to better detect other putative abnormal processing events which are hypothesized to potentially either compete with secretase for limited substrate, or occur at much lower frequency than secretase and whose effects may be otherwise masked by the mass action of secretase.

Third, secretase-cleavable and -noncleavable APP substrates would provide probes with which to investigate cellular posttranslational modifications to APP in an attempt to determine the potential influence on normal secretase and abnormal BAP "clipping" activities. These areas include, among others, the consideration of various known APP point mutations, contribution by different cell/tissue types (normal- or AD-specific), the Kunitz Protease Inhibitor domain present in APP-770 and -751 isoforms, APP phosphorylation and APP glycosylation.

Fourth, the ability to detect specific APP proteolytic events, either the normal secretase or the abnormal BAP-generating activities, would enable the use of strategies which use phenotypic rescue as a marker for the cloning of potentially relevant and interesting proteases in tissue culture systems.

Overview of the APP-REP Strategy

To study secretase and BAP-generating pathways, portions of APP cDNA clones are used to engineer a panel of APP-REPorter (APP-REP) plasmids to express "marked" proteins representing each of the APP isoforms (and other APP/BAP sequence alterations; see below) in cultured cells. The system utilizes the marker Substance-P (SP) and Met-Enkephalin (ME) which are strategically placed, respectively, on amino- and carboxy-terminal sides of BAP. Proteolytic cleavage of APP-REP target substrate is determined by the electrophoretic sizing of resulting proteolytic fragments and immunological detection of APP-specific and SP and ME reporter epitopes. Deletion of a large central portion of APP sequence also makes APP-REP readily distinguishable from the endogenous APP isoforms based on size. Moreover, the resolution of detecting proteolytic cleavage at different positions within the APP-REP substrate protein is enhanced by working with shorter target substrates. Approximate location of cleavage is determined initially by fragment sizing and epitope mapping; the exact cleavage site is later determined by peptide mapping of affinity/HPLC purified fragments and sequencing of peptide ends.

Plasmids also are derived from these constructs for developing similar strategies to express APP-REP protein in cell free reticulocyte transcription-translation and bacterial systems. Mutation of APP-REP secretase/BAPase cleavage site (by sequence substitution, deletion or FAD mutations) can reveal putative proteolytic activities associated with BAP formation including amino- and carboxy-BAPase activities which are predicted to result in altered product fragments lengths.

FIRST SERIES OF EXPERIMENTS

Bacterial Strains and Transformation

Transformation of commercially available frozen competent bacteria, maintenance and selection of transformants is according to the manufacturer. Strains HB101, DH5a or JM109 (Gibco-BRL) are used for the construction of APP-REP in pSK(+) (Stratagene, La Jolla, CA) and pSL 301 (Invitrogen, San Diego, CA). APP-REP is subsequently subcloned into the eucaryotic expression vector pcDNA-1-neo and amplified in MC1061/P3 (Invitrogen, San Diego, CA).

Plasmid Construction

A cassette approach is used to independently construct portions of the APP-REP plasmid (Figure 2). The N-terminal partial includes APP sequences through the Substance P (SP) epitope, while the carboxy-terminal (C-terminal) partial includes BAP (or sequence variations of BAP) through the Metenkephalin (ME) epitope (Figure 1). Plasmid encoding the N-terminal cassette (pCLL935) is constructed by ligating the EcoRI-XhoI fragment derived from APP-751 cDNA to a short synthetic XhoI-HindIII fragment encoding Substance P (amino acid 1-11). This product is then ligated into the EcoRI and HindIII sites of pSK(+). Plasmid encoding the carboxy-terminal (C-terminal) cassette (pCLL947) is constructed by cloning into the HindIII-BamHI sites of pSL301 a fragment containing BAP sequences which is amplified by polymerase chain reaction. The fragment features a novel 5'-HindIII site beginning at lysine 638 of APP-751, native BAP through APP C-terminal sequences, and a C-terminal fusion including the Metenkephalin epitope followed by a stop translation codon and a BamHI site. The resulting pSL301 HindIII-SalI fragment (including the HindIII-BamHI coding region plus BamHI-SalI polylinker sequences) is then isolated and ligated to the N-terminal cassette by subcloning into the HindIII-SalI sites of the SK(+)-based, CMV promoter driven, eukaryotic expression vector pcDNA-1-neo (pCLL601), whose polylinker is modified to accommodate the APP-REP fragment (pCLL602). Polylinker modification involves the substitution of the HindIII-XbaI fragment with a synthetic one which restores HindIII, destroys XbaI and introduces novel BamHI-XabI-Xho-SalI sites.

Tissue Culture Lines

All cells are obtained from American Type Culture Collection and maintained according to their recomm ndation. They include SV40-transformed African Green monkey kidney COS-1 cells (CRL 1650) for transient expression and Chinese hamster ovary CHO-1C6 (CRL 1973) for stable expression systems.

Transfection Procedure

Cells are seeded at a density of 2-3 X 10^5/100 mm dish and transfected using Lipofectin (Gibco-BRL, Grand Island, NY) when ~75% confluent. Plasmid DNA (0.5-4 mg) is diluted in 450 ml of Opti-MEM (Gibco-BRL, Grand Island, NY), mixed with 450 ml containing 75-100 ml Lipofectin and the mixture incubated at room temperature for 20-30 minutes. Addition of DNA-Lipofectin mixture to cells, recovery phase and G418 selection (Gibco-BRL), when applicable, are according to the manufacturer's protocol. Cells and conditioned medium are harvested at 48-72 hours following transfection for assay of APP-REP expression.

Antisera

APP-specific antisera:anti-N-terminal APP, mouse monoclonal 22C11 (Boehringer-Mannheim Biochemicals, Indianapolis, IN) raised against a recombinant fusion protein expressing APP-695 (epitope mapped to aa 60-100); anti-KPI rabbit polyclonal, raised against recombinant protein encoded by the Hinfl fragment derived from APP-770; and anti-APP C-terminal rabbit polyclonal M3, raised against synthetic APP peptides corresponding to APP-770 amino acid residues 649-671. Reporter-specific antisera:anti-substance P, rabbit polyclonal, purchased from Peninsula, Belmont, CA; and anti-Met-enkephalin, rabbit polyclonal, purchased from Cambridge, Wilmington, DE.

Preparation of Radiolabeled APP-REP and Extraction from Conditioned Medium and Cell Lysates

APP-REP proteins transiently expressed in exponentially growing adherent cells (~4 x 10^6) are radiolabeled by metabolic incorporation of [^{35}S]-methionine as follows. Cell monolayers are washed twice with prelabeling medium (methionine-free D-HEM supplemented with glutamine, sodium pyruvate, antibiotics and 1% dialyzed fetal bovine serum (Gibco-BRL) and incubated for 15 minutes to 4 hours in prelabeling medium containing 150-450 uCi[^{35}S]-methionine (Amersham, 800Ci/mmol). If chased with cold methionine, the medium is removed following the pulse, the monolayer is washed with prelabeling medium and replaced with 3 ml of the same containing 1 mM cold methionine.

The conditioned medium is recovered following radiolabeling by aspiration from plates and cell debris removed by centrifugation for 10 minutes at 4°C (~300xg). Conditioned medium is immediately supplemented with protease inhibitors (pepstatin A, 50 ug/ml; leupeptin, 50 ug/ml; aprotinin, 10 ug/ml; EDTA, 5 mM; PMSF, 0.25 mM) and immunoprecipitation buffer (IPB; Sisodia et al., 1990) for protein analysis. Briefly, 3 ml of CM is supplemented with 0.75 ml 5X IPB (250 mM Tris, pH 6.8; 750 mM NaCl; 25 mH EDTA; 2.5% Nonidet P40; 2.5% sodium deoxycholate) and incubated for 20 minutes at 4°C prior to use.

Lysates are prepared by washing the labeled cell monolayer twice with 5 ml pre-labeling medium and directly extracting cells in plates at 4° C with 3.75 ml 1X IPB (including protease inhibitors). Cells are scraped into the buffer, incubated for 20 minutes at 4°C and lysates clarified of cellular debris by centrifugation for 20 minutes at 10,000xg.

For radioiodination of cell surface proteins, monolayers are chilled on ice, washed 3 times with 5 ml ice cold PBS and labeled at room temperature for 10 minutes following the addition of: 5 ml PBS containing 0.2 mCi Iodine-125 (NEZ-033A, New England Nuclear), 0.25 ml lactoperoxidase (1 mg/ml distilled water, Sigma), 10 ul of hydrogen peroxide solution (freshly prepared by diluting 10 ml of 30% stock in 10 ml of PBS) added at 0, 3, 6, and 9 minutes of iodination. At 10 minutes, the supernatant is removed and cells gently washed with 10 ml of ice cold PBS (containing 10 mM NaI). Four ml of PBS is added, and CM and cell lysates are prepared as above.

Immunoprecipitation Analysis

Aliquots of radiolabeled lysate or conditioned medium representing 4-8x10^5 cells are thawed on ice, supplemented with protease inhibitors (see above), boiled for 3 minutes in 0.35% SDS and chilled on ice. Samples are preincubated on a shaker for 1.5 hours at 4°C with 2-10 ul 2X of preimmune (or normal rabbit) serum and 2 mg Protein A-Sepharose (Sigma; prepared in 1X IPB), and insoluble immune removed by contrifugation. APP-or reporter epitope-specific antisera (0.1-10 ul) and 2 mg Prot in A-S pharose wer similarly added and incubated overnight. Specific immune complexes were pr cipitated, washed 4 times with 0.25 ml 1 X IPB (with protease inhibitors), extracted with 20 ul Laemmli sample buffer (Laemmli (1970) Nature 227:680-685), boiled for 3 minutes and fractionated by electrophoresis on SDS-polyacrylamide-tris-glycine (Bio-Rad Laboratories, Richmond, VA) or SDS-polyacrylamide-tris-tricine Daiichi (Int grated Separation Systems, Natick, MA) gels. Gels are then treated with Enlightening Autoradiographic Enhanc r (New

England Nuclear, NEF-974) and dried in vacuo with heat and exposed to Kodak X-AR film at -70°C.

Western (Immunoblot) Analysis

Lysate or 10X concentrated conditioned medium (Centricon 30 microconcentrator; Amicon, Beverly, MA) representing 4-8x10^5 cells are supplemented with an equal volume of 2X Laemmli sample buffer, boiled for 2 minutes, fractionated by electrophoresis on SDS-polyacrylamide-tris-glycine (Bio-Rad, XX) or SDS-polyacrylamide-tris-tricine Daiichi (Integrated Separation Systems, Natick, MA) gels and transblotted (Semi-Phor, Hoefer Instruments, San Francisco, CA) to Immobilon-P membrane (Millipore, Bedford, MA). Membranes are pre-blocked in 10 ml 5% non-fat dry milk/PBST (PBS with 0.02% Tween-20) for 45 minutes at room temperature prior to overnight incubation at 4°C with primary antisera (in fresh pre-blocking solution). Blots are then washed, incubated with secondary antibody, washed and developed for horseradish peroxidase activity as described (ECL Luminol Kit; Amersham, Arlington Heights, IL).

Peptide Mapping and Determination of the Site of Proteolytic Cleavage by Peptide Sequencing

The secretase clip site is determined essentially as described (Wang et al., (1991) J. Biol. Chem. 266:16960-16964). Approximately 1X10^6 CHO cells stably expressing APP-REP are seeded in each 150 mm dish containing DMEM (complete with 200 ug/ml G418) and incubated for 36 hours. Cells are washed, preincubated for 6 hours in serum-free medium [MCDB 302 supplemented with antibiotics, L-glutamine (292 mg/l) and proline 12 mg/l (Sigma) to remove serum components, washed, and incubated for another 72 hours in fresh serum-free media.

Serum-free conditioned medium was pooled and cell debris is removed by centrifugation (10 minutes at 300xg, then 30 minutes at 100,000xg) and concentrated by acetone precipitation and fractionated by FPLC. Conditioned medium concentrate is loaded on an anion exchange column (Mono Q; source) and protein is eluted in 20 mM Tris (pH 7.4) over a 0-1M NaCl gradient. Fractions containing secreted APP are identified by immunoblotting (monoclonal antibody 22C11) and relevant samples pooled, desalted (NP-5 column; Pharmacia, Piscataway, NJ) and concentrated. Proteins are then denatured, treated with cyanogen bromide (in 10% trifluoroacetic acid) and peptides separated by high performance liquid chromatography (Vydac C_{18} reverse-phase) attached to a FAB-MS unit. Relevant peaks derived from APP-REP 751 and APP-REP BAP $_{11-28}$ are identified by locating those peaks uncommon to both proteins. The C-terminal peptides derived from APP-REP BAP $_{11-28}$ (predicted 14 amino acid) and APP-REP 751 (predicted 17 amino acid) are sequenced (MilliGen solid phase peptide sequencer; Millipore, Burlington, MA).

EXPERIMENTAL RESULTS

Characterization of APP-REP Expression by Epitope Mapping

The APP-REP strategy (Figure 1) is system for the expression of marked APP proteins in tissue culture cells in order to characterize the proteolytic cleavage events. The deletion of 276 amino acid portion distinguishes the construct of this invention from endogenously expressed APP on the basis of size, and is predicted to increase the resolution of APP-REP fragments resulting from the proteolytic cleavage by secretase or other amyloidogenic, BAP-generating cleavage events. Substance P and Met-enkephalin marker epitopes strategically placed on either side of BAP enable the immunological detection of N- and C-terminal fragments, respectively, which result from proteolytic cleavage of APP-REP substrate.

APP-REP protein transiently expressed in COS-1 cells has been radiolabeled by metabolic incorporation of [^{35}S]-methioninein a 60 minute pulse, immunoprecipitated with antisera, and size fractionated by gel electrophoresis as demonstrated in Figure 3. Immunoprecipitation with a panel of APP- and APP-REP-specific antisera which recognize epitopes mapping at various positions along APP-REP, reveals the presence of 2 proteins of ~63 kDa in cell lysates (including cytoplasmic and membrane associated proteins) as shown in Figure 3. The specific detection by antisera directed against the KPI domain, the carboxy-terminus of APP (M3, Figure 3A) and Met-enkephalin, as well as by the N-terminal 22C11 monoclonal in Western blot analysis (data not shown), suggests that both bands repres nt th full-length APP-REP protein. Although the 492 amino acid APP-REP is predicted to display a mobility of ~49-54 KdA, the larger 63 and 76 kDa proteins are xpected based on previous observations attributing the aberrant migration properties of APP, putatively to post-translational modification like tyrosine-sulfation, glycosylation and phosphorylation (Dyrks et al., (1988) EHSO J. 7:949-957; Weidemann et al., (1989) Cell 57:115-126.

Analysis of the conditioned medium (CM) collected from those same cells above indicates that an N-terminal fragment of APP-REP is rel ased into the CM. Figure 3B reveals a shorter ~67 kDa fragment immunoprecipitable from CM with KPI and SP antisera (and the 22C11 monoclonal by Western analysis), but not with several C-terminal APP or ME antisera. These data are consistent with the observations (Selkoe et al., (1988) P.N.A.S. 86:6338-6342; Palmert et al., (1989 a) P.N.A.S. U.S.A. 85:7341-7345), b) indicating that APP is a substrate for the proteolytic cleavage resulting in the secretion of an N-terminal fragment into CM, and a short membrane associated C-terminal fragment.

Pulse-Chase Analysis Reveals the Precursor/Product Relationship Between Cell Associated and Secreted Derivatives of APP-REP

To show that APP-REP undergoes post-translational modification accounting for the 2 cell associated proteins, and that the N-terminal APP-REP fragment released into CM is derived from one of these precursors, radiolabeled APP-REP is with a short 15 minute pulse and collected both cell lysates and CM at various chase intervals as shown in Figure 4. Immunoprecipitation analysis reveals that APP-REP initially migrates at ~63 kDa and is rapidly "chased" up to ~76 kDa with conversion rate of less than 10-15 minutes (Figure 4A; also see Figure 5C for quantitative analysis), an observation which is consistent with the notion that APP-REP, like APP, is substrate for posttranslational modifications.

The ~76 kDa APP-REP band (cell lysate) rapidly disappears (t $^{1/2}$ ~20 minutes) (Figure 4A and 5C), followed by the appearance of a shorter ~67 kDa band in the CM (Figure 4B and 5C). The released ~67 kDa fragment accumulates rapidly and is relatively long lived (t 1/2 > 8 hours). The temporal pattern of intracellular APP-REP depletion, accumulation of a shorter ~67 kDa protein in CM, and the recognition of this protein only by antisera raised against N-terminal epitopes, is consistent with proteolytic cleavage of APP-REP which is similar to the normal, non-amyloidogenic, "secretase" activity which results in the release of an N-terminal APP fragment (Sisodia et al., (1990) Science 248:492-495.

Expression of APP-REP Derivatives Containing Altered BAP Sequences Does Not Prevent Proteolytic Cleavage

In an attempt to engineer non-cleavable substrates for secretase, APP-REP proteins are expressed (Figure 5A) either lacking the secretase "cleavage/recognition site" putatively encompassed by aa residues BAP 11-28 ($BAP_{\Delta 11-28}$pCLL604), or representing the BAP point mutation found in patients with HCHWA-D (BAP $_{E22Q}$,pCLL603). The construct representing the BAPE22Q mutation results in secretion of an N-terminal fragment indistinguishable from the APP-REP protein (Figure 5C). Deletion of extracellular, juxtamembranous 18 aa (BAP_{Δ} 11-28), however, still results in the secretion of an N-terminal APP-REP fragment into the CM (Figure 5B). A slightly faster migration of fragment derived from the deletion construct pCLL604 in comparison to that of wild-type pCLL602, is consistent with the 18 aa deletion and a corresponding loss of ~2 kDa (Figure 5C). Pulse-chase analyses (Figure 5D) indicate that expression of full-length precursor by each construct, proteolytic cleavable and the release of fragment into CM is both qualitatively and quantitatively similar to that of the wild-type APP-REP sequence. Chinese hamster ovary (CHO) cells stably expressing APP-REP display results similar to that of transiently expressing COS-1 cells (Figure 5E). Collectively, these data suggest that the cleavage in each case may be the result of similar biochemical events despite the difference in juxtamembranous sequences (Figure 5A).

Full-Length APP-REP Proteins Are Associated with Plasma Membrane Prior to Cleavage

In preliminary experiments, detection of the amino-terminal APP-REP fragment in CM and not in cell lysates, suggests that the putative secretase activity might be plasma membrane-associated. One prediction of this notion is that an N-terminal portion of APP-REP might be (partially) localized to the extracellular environment prior to cleavage. In order to test this hypothesis, CHO cells stably expressing APP-REP (pCLL602) are subjected to lactoperoxidase-catalyzed iodination to radiolabel only extracellular proteins associated with the cell surface, and CM and cell lysates were analyzed immediately following iodination or after a 10 minute incubation. Pr sence of the ~76 kDa APP-REP band in cell lysate should indicate that at least a portion of full-length APP-REP is poised extracellularly in association with cell membran . Detection of both, a reduced fraction of th ~76 kDa band in the cell lysate and a corresponding incr ased fraction of ~67 kDa fragment in CM following the "release" incubation, would suggest that the xtracellular portion of APP-REP is cleaved.

Peptide Sequencing to Determine the site of Proteolysis

Fragment secreted into serum-free media derived from CHO cells stably expressing APP-REP with wild-type or BAP 11-28 sequences has been analyzed to determine the actual site of proteolytic cleavage as shown in Figure 6. Peptide mapping by tryptophan-specific cleavage with BNPS-skatole is used to roughly determine the approximate position of cleavage in each molecule. Western blot analysis using SP antisera following BNPS-skatole treatment (Figure 6B) reveals fragments whose lengths of ~10.5 and ~9.5 kDa, corresponding to wild type and BAP $_{11-28}$respectively, confirming that cleavage occurs in the C-terminal portion of the PN-2-like protein as expected (Figure 6A). To determine the actual position of cleavage, secreted fragment is partially purified, treated with cyanogen bromide and relevant C-terminal peptides derived from APP-REP wild type.

DISCUSSION

The examined the expression of a truncated form of APP-751, namely APP-REP 751 (pCLL602) is examined and its normal cleavage by secretase. Comparison of the nontransfected cells and those transfected with APP-REP 751, in both COS-1 transient and CHO stable expression systems, show the production of shorter secreted protein derived from APP-REP. Furthermore, upon a prolonged exposure of the fluorogram only one band is observed in condition medium. Epitope mapping with antibodies to N- and C-terminal domains of APP-REP and amino acid sequencing suggest post-translational cleavage at a site similar to that reported for intact APP protein and other truncated APP constructs similar to that reported for intact APP protein and other truncated APP constructs. Pulse-chase experiments reveal post-translational modifications, believed to be similar to those described for the intact APP protein, in which a single ~63 kDa product is chased up to ~76 kDa in the first 30 minutes. Appearance of the ~76 kDa cell membrane associated protein precedes the release of a ~67 kDa product into the CM. The released form, which is not observed in the cell lysate fraction, steadily accumulates in the conditioned medium well after the ~76 kDa band has begun to disappear suggesting a precursor-product relationship. These data indicate that the APP-REP protein is a good representation of the naturally occurring APP with respect to post-translational synthesis, processing, and stability in a tissue culture system.

Epitope mapping of APP-REP 751 mutants suggest that BAP $_{E22Q}$,as well as the BAP$_{\Delta11-28}$deletion constructs, are initially expressed as larger proteins of predicted lengths which subsequently are cleaved to release N-terminal fragments into the CM. The pulse-chase experiments indicate the cell-associated and secreted forms accumulate with similar kinetics.

TABLE 1

Construction of APP-REP Partials

A. pSK(+) Amino-Terminal Constructs: Cloning of APP Isoform and Reporter Epitope (EcoRI-HindIII Fragments)

Plasmid Name	APP Isoform (EcoRI-XhoI Fragment)	Reporter Epitope (XhoI-HindIII Fragment)
pCLL983	APP-695	Substance P*
pCLL935	APP-751	Substance P
pCLL934	APP-770**	Substance P
pCLL913	APP-770#	Substance P

Notes:

* Substance P is a peptide containing 11 residues with the amino acid sequence of RPKPQQFFGLM.

** 5' untranslated sequences derived from the shorter APP-770 cDNA form.

\# 5' untranslated sequences derived from the longer APP-751 cDNA form.

B. pSL301 Carboxy-Terminal Constructs: Cloning of BAP-Encoding APP Reporter Epitope Fusions (HindIII-BamHI/SalI Fragment)

Plasmid Name	Met-Enkephalin (ME) Fusion at end of:	Name of Variation
pCLL947	Full-Length APP	APP-BAP-APP-ME
pCLL914	Transmembrane Domain	APP-BAP-TM-ME
pCLL937	BAP	APP-BAP-ME

TABLE 1

Construction of APP-REP Partials

(Continued)

C. pSL301 Carboxy-Terminal Full-Length APP-ME Constructs: Introduction of Mutations in BAP (HindIII-BamHI/SalI Fragment)

Plasmid Name	Met-Enkephalin Fusion at End of:	Name of Variation
pCLL949	E to Q substitution at BAP aa#22	BAP22EQ
pCLL957	G to A substitution at BTaa#10, deletion of BAP AA#11-28 and creation of NdeI site	BAP-vaa11-28

TABLE 2

Assembly of APP-REP Full-Length Constructs Containing Substance P and Met-Enkephalin Reporter Epitopes and BAP or a Variation of BAP

Plasmid Name	Construct Name/Variation	Plasmid (N-Terminus)	Restriction Fragment (C-Terminus)
pCLL618	APP-REP-695	pCLL983	pCLL947
pCLL964	APP-REP-751	pCLL935	pCLL947
pCLL962	APP-REP-770	pCLL934	pCLL947
pCLL619	APP-REP-695/$BAP_{E\ to\ Q}$	pCLL983	pCLL949
pCLL989	APP-REP-751/$BAP_{E\ to\ Q}$	pCLL935	pCLL949
pCLL987	APP-REP-770/$BAP_{E\ to\ Q}$	pCLL934	pCLL949
pCLL620	APP-REP-695/$BAP_{aa11-28}$	pCLL983	pCLL957
pCLL990	APP-REP-751/$BAP_{aa11-28}$	pCLL935	pCLL957
pCLL988	APP-REP-770/$BAP_{aa11-28}$	pCLL934	pCLL957

TABLE 3
Subcloning of APP-REP Full-Length Constructs and Human Growth Hormone (hGH) into pcDNA-1-Neo[XS]

Plasmid Name	Construct Name (in pcDNA-1-neo)	Source of Insert
pCLL600	pcDNA-1-neo-hGH	pOGH*
pCLL601	pcDNA-1-neo[XS]	Synthetic Fragment**
pCLL602	APP-REP-751	pCLL964
pCLL603#	APP-REP-751/BAP$_{E\ to\ Q}$	pCLL989
pCLL604#	APP-REP-751/BAP$_{aa11-28}$	pCLL990
pCLL605	APP-REP-770	pCLL962
pCLL606	APP-REP-770/BAP$_{E\ to\ Q}$	pCLL987
pCLL607	APP-REP-770/BAP$_{aa11-28}$	pCLL988

Notes:

* The HindIII-EcoRI (blunt-ended) fragment encoding hGH sequences of pOHG (Nichols Diagnostics) was subcloned into the HindIII-EcoRI (blunt-ended) sites of pcDNA-1-neo.

** The HindIII-XbaI fragment of the pcDNA-1-neo polylinker was replaced with a synthetic fragment which destroyed the original XbaI site and introduced several unique sites (HindIII-BamHI-XbaI-XhoI-SalI).

\# Also created by an alternative strategy using the same pSK(+) plasmids.

TABLE 4
"Secretase-Minus" APP-REP Constructs
Engineered by Oligonucleotide-Directed Mutagenesis

Plasmid Name	Mutation Identity	Mutated BAP Sequence Compared to Wild Type								Percent** Secretion
		14	15	16	|	17	18	19	20	
pCLL602	BAP*	CAT	CAA	AAA	|	TTG	GTG	TTC	TTT	100
		H	Q	K	|	L	V	F	F	
pCLL608	BAP-16KE	CAT	CAA	GAG	|	TTG	GTG	TTC	TTT	0
		H	Q	E	|	L	V	F	F	
pCLL609	BAP-16KV	CAT	CAA	GTG	|	TTG	GTG	TTC	TTT	10-20
		H	Q	V	|	L	V	F	F	
pCLL610	BAP-19FP	CAT	CAA	AAA	|	TTG	GTG	CCG	TTT	10-20
		H	Q	K	|	L	V	P	F	

Notes:

* Wild-type BAP

* % secretion relative to wild type BAP sequence as determined by Sisodia.

TABLE 5
APP-REP Constructs Modeling APP Mutations
Associated with Diseases Involving BAP Deposition

APP "717" MUTATIONS

// APP Transmembrane Domain /

// [BAP]

		711	712	713	714	715	716	717	718	719
		[40	41	42]						
pCLL602	APP*	GTC	ATA	GCG	ACA	GTG	ATC	GTC	ATC	ACC
		V	I	A	T	V	I	V	I	T
pCLL611	717VI**	GTC	ATA	GCG	ACA	GTG	ATC	ATC	ATC	ACC
		V	I	A	T	V	I	I	I	T
pCLL612	717VG@	GTC	ATA	GCG	ACA	GTG	ATC	GGC	ATC	ACC
		V	I	A	T	V	I	G	I	T
pCLL613	717VF$	GTC	ATA	GCG	ACA	GTG	ATC	TTC	ATC	ACC
		V	I	A	T	V	I	F	I	T

TABLE 5 (continued)

DUTCH DISEASE : V (secretase clip)

	686	687	:	688	689	690	691	692	693	694
	[15	16	:	17	18	19	20	21	22	23]
pCLL602 BAP*	CAA	AAA	:	TTG	GTG	TTC	TTT	GCG	GAA	GAT
	Q	K	:	L	V	F	F	A	E	D
pCLL603 BAP-22EQ#	CAA	AAA	:	TTG	GTG	TTC	TTT	GCA	CAA	GAT
pCLL606#	Q	K	:	L	V	F	F	A	Q	D

Notes:

APP-REP-751 and -770 derived BAP-22EQ constructs.

** Goate *et al.* (1991) Nature, 349:704-706; Yoshioka *et al.* (1991) BBRC 178:1141-1146; Naruse *et al.* (1991) Lancet 337:978-979.

@ Chartier-Harlin *et al.* (1991) Nature 353:844-846.

$ Murrell *et al.* (1991) Science 254:97-99.

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

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Gln Lys Leu Val Phe Phe Ala Gln Asp
  1                 5
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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: American Cyanamid Company
(B) STREET: 1937 West Main Street
(C) CITY: Stamford
(D) STATE: Connecticut
(E) COUNTRY: U.S.A
(F) POSTAL CODE (ZIP): 06904-0060

(ii) TITLE OF INVENTION: Novel Amyloid Precursor Proteins and Methods of Using Same

(iii) NUMBER OF SEQUENCES: 27

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 93105718

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1721 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 196..1671

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTGGGG ATCCGCTCTA GAACTAGTGG ATCCCCCGGG CTGCAGGAAT TCGGGGGGGG 60

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CAGCGGTAGG CGAGAGCACG CGGAGGAGCG TGCGCGGGGC CCCGGGAGAC GGCGGCGGTG     120

GCGGCGCGGG CAGAGCAAGG ACGCGGCGGA TCCCACTCGC ACAGCAGCGC ACTCGGTGCC     180

CCGCGCAGGG TCGCG ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG GCC GCC      231
                 Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala
                  1               5                  10

TGG ACG TCT CGG GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT GGC CTG       279
Trp Thr Ser Arg Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu
         15                  20                  25

CTG GCT GAA CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC ATG CAC       327
Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His
     30                  35                  40

ATG AAT GTC CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG ACC AAA       375
Met Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys
 45                  50                  55                  60

ACC TGC ATT GAT ACC AAG GAA GGC ATC CTG CAG TAT TGC CAA GAA GTC       423
Thr Cys Ile Asp Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val
                 65                  70                  75

TAC CCT GAA CTG CAG ATC ACC AAT GTG GTA GAA GCC AAC CAA CCA GTG       471
Tyr Pro Glu Leu Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val
             80                  85                  90

ACC ATC CAG AAC TGG TGC AAG CGG GGC CGC AAG CAG TGC AAG ACC CAT       519
Thr Ile Gln Asn Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His
         95                 100                 105

CCC CAC TTT GTG ATT CCC TAC CGC TGC TTA GTT GGT GAG TTT GTA AGT       567
Pro His Phe Val Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser
    110                 115                 120

GAT GCC CTT CTC GTT CCT GAC AAG TGC AAA TTC TTA CAC CAG GAG AGG       615
Asp Ala Leu Leu Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg
125                 130                 135                 140

ATG GAT GTT TGC GAA ACT CAT CTT CAC TGG CAC ACC GTC GCC AAA GAG       663
Met Asp Val Cys Glu Thr His Leu His Trp His Thr Val Ala Lys Glu
                145                 150                 155

ACA TGC AGT GAG AAG AGT ACC AAC TTG CAT GAC TAC GGC ATG TTG CTG       711
Thr Cys Ser Glu Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu
            160                 165                 170

CCC TGC GGA ATT GAC AAG TTC CGA GGG GTA GAG TTT GTG TGT TGC CCA       759
Pro Cys Gly Ile Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro
        175                 180                 185
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CTG GCT GAA GAA AGT GAC AAT GTG GAT TCT GCT GAT GCG GAG GAG GAT      807
Leu Ala Glu Glu Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp
    190                 195                 200

GAC TCG GAT GTC TGG TGG GGC GGA GCA GAC ACA GAC TAT GCA GAT GGG      855
Asp Ser Asp Val Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly
205                 210                 215                 220

AGT GAA GAG AAA GTA GTA GAA GTA GCA GAG GAG GAA GAA GTG GCT GAG      903
Ser Glu Glu Lys Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu
                225                 230                 235

GTG GAA GAA GAA GAA GCC GAT GAT GAC GAG GAC GAT GAG GAT GGT GAT      951
Val Glu Glu Glu Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp
            240                 245                 250

GAG GTA GAG GAA GAG GCT GAG GAA CCC TAC GAA GAA GCC AGA GAG AGA      999
Glu Val Glu Glu Glu Ala Glu Glu Pro Tyr Glu Glu Ala Arg Glu Arg
        255                 260                 265

ACC ACC AGC ATT GCC ACC ACC ACC ACC ACC ACC ACA GAG TCT GTG GAA     1047
Thr Thr Ser Ile Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu
    270                 275                 280

GAG GTG GTT CGA GAG GTG TGC TCT GAA CAA GCC GAG ACG GGG CCG TGC     1095
Glu Val Val Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys
285                 290                 295                 300

CGA GCA ATG ATC TCC CGC TGG TAC TTT GAT GTG ACT GAA GGG AAG TGT     1143
Arg Ala Met Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys
                305                 310                 315

GCC CCA TTC TTT TAC GGC GGA TGT GGC GGC AAC CGG AAC AAC TTT GAC     1191
Ala Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp
            320                 325                 330

AGA GAA GAG TAC TGC ATG GCC GTG TGT GGG AGC GCC ATT CCT ACA ACA     1239
Arg Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr
        335                 340                 345

GCA GCC AGT ACC CCT GAT GCC GTT GAC AAG TAT CTC GAG CGG CCC AAG     1287
Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Arg Pro Lys
    350                 355                 360

CCC CAG CAG TTC TTT GGC CTG ATG GGA AGC TTG ACA AAT ATC AAG ACG     1335
Pro Gln Gln Phe Phe Gly Leu Met Gly Ser Leu Thr Asn Ile Lys Thr
365                 370                 375                 380

GAG GAG ATC TCT GAA GTG AAG ATG GAT GCA GAA TTC CGA CAT GAC TCA     1383
Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser
                385                 390                 395
```

```
GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC TTT GCA GAA GAT GTG        1431
Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val
            400                 405                     410

GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC        1479
Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val
        415                 420                     425

ATA GCG ACA GTG ATC GTC ATC ACC TTG GTG ATG CTG AAG AAG AAA CAT        1527
Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys His
    430                 435                     440

TAC ACA TCC ATT CAT CAT GGT GTG GTG GAG GTT GAC GCC GCT GTC ACC        1575
Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr
445                 450                     455                     460

CCA GAG GAG CGC CAC CTG TCC AAG ATG CAG CAG AAC GGC TAC GAA AAT        1623
Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn
                465                     470                     475

CCA ACC TAC AAG TTC TTT GAG CAG ATG CAG AAC TAT GGG GGC TTC ATG        1671
Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn Tyr Gly Gly Phe Met
            480                 485                     490

TAGGATCCAT ATATAGGGCC CGGGTTATAA TTACCTCAGG TCGACCTAGA                 1721
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 492 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ser Arg
 1               5                  10                  15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80
```

```
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                 85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Glu Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Arg Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
    290                 295                 300

Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305                 310                 315                 320

Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Arg Glu Glu Tyr
                325                 330                 335

Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser Thr
            340                 345                 350

Pro Asp Ala Val Asp Lys Tyr Leu Glu Arg Pro Lys Pro Gln Gln Phe
        355                 360                 365
```

```
Phe Gly L u Met Gly Ser Leu Thr Asn Il  Lys Thr Glu Glu Il  S r
    370                 375                 380

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
385                 390                 395                 400

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
                405                 410                 415

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
            420                 425                 430

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys His Tyr Thr Ser Ile
        435                 440                 445

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
    450                 455                 460

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
465                 470                 475                 480

Phe Phe Glu Gln Met Gln Asn Tyr Gly Gly Phe Met
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGTTTCCTCG GCAGCGGTAG GCGAGAGCAC GCGGAGGAGC GTGCGCGGGG CCCCGGGAGA    60
CGGCGGCGGT GGCGGCGCGG GCAGAGCAAG GACGCGGCGG ATCCCACTCG CACAGCAGCG   120
CACTCGGTGC CCCGCGCAGG GTCGCGATGC TGCCCGGTTT GGCACTGCTC CTGCTGGCCG   180
CCTGGACGGC TCGGGCGCTG GAGGTACCCA CTGATGGTAA TGCTGGCCTG CTGGCTGAAC   240
```

CCCAGATTGC CATGTTCTGT GGCAGACTGA ACATGCACAT GAATGTCCAG AATGGGAAGT 300
GGGATTCAGA TCCATCAGGG ACCAAAACCT GCATTGATAC CAAGGAAGGC ATCCTGCAGT 360
ATTGCCAAGA AGTCTACCCT GAACTGCAGA TCACCAATGT GGTAGAAGCC AACCAACCAG 420
TGACCATCCA GAACTGGTGC AAGCGGGGCC GCAAGCAGTG CAAGACCCAT CCCCACTTTG 480
TGATTCCCTA CCGCTGCTTA GTTGGTGAGT TTGTAAGTGA TGCCCTTCTC GTTCCTGACA 540
AGTGCAAATT CTTACACCAG GAGAGGATGG ATGTTTGCGA AACTCATCTT CACTGGCACA 600
CCGTCGCCAA AGAGACATGC AGTGAGAAGA GTACCAACTT GCATGACTAC GGCATGTTGC 660
TGCCCTGCGG AATTGACAAG TTCCGAGGGG TAGAGTTTGT GTGTTGCCCA CTGGCTGAAG 720
AAAGTGACAA TGTGGATTCT GCTGATGCGG AGGAGGATGA CTCGGATGTC TGGTGGGGCG 780
GAGCAGACAC AGACTATGCA GATGGGAGTG AAGACAAAGT AGTAGAAGTA GCAGAGGAGG 840
AAGAAGTGGC TGAGGTGGAA GAAGAAGAAG CCGATGATGA CGAGGACGAT GAGGATGGTG 900
ATGAGGTAGA GGAAGAGGCT GAGGAACCCT ACGAAGAAGC CACAGAGAGA ACCACCAGCA 960
TTGCCACCAC CACCACCACC ACCACAGAGT CTGTGGAAGA GGTGGTTCGA GTTCCTACAA 1020
CAGCAGCCAG TACCCCTGAT GCCGTTGACA AGTATCTCGA GACACCTGGG GATGAGAATG 1080
AACATGCCCA TTTCCAGAAA GCCAAAGAGA GGCTTGAGGC CAAGCACCGA GAGAGAATGT 1140
CCCAGGTCAT GAGAGAATGG GAAGAGGCAG AACGTCAAGC AAAGAACTTG CCTAAAGCTG 1200
ATAAGAAGGC AGTTATCCAG CATTTCCAGG AGAAAGTGGA ATCTTTGGAA CAGGAAGCAG 1260
CCAACGAGAG ACAGCAGCTG GTGGAGACAC ACATGGCCAG AGTGGAAGCC ATGCTCAATG 1320
ACCGCCGCCG CCTGGCCCTG GAGAACTACA TCACCGCTCT GCAGGCTGTT CCTCCTCGGC 1380
CTCGTCACGT GTTCAATATG CTAAAGAAGT ATGTCCGCGC AGAACAGAAG GACAGACAGC 1440
ACACCCTAAA GCATTTCGAG CATGTGCGCA TGGTGGATCC CAAGAAAGCC GCTCAGATCC 1500
GGTCCCAGGT TATGACACAC CTCCGTGTGA TTTATGAGCG CATGAATCAG TCTCTCTCCC 1560
TGCTCTACAA CGTGCCTGCA GTGGCCGAGG AGATTCAGGA TGAAGTTGAT GAGCTGCTTC 1620
AGAAAGAGCA AAACTATTCA GATGACGTCT TGGCCAACAT GATTAGTGAA CCAAGGATCA 1680
GTTACGGAAA CGATGCTCTC ATGCCATCTT TGACCGAAAC GAAAACCACC GTGGAGCTCC 1740
TTCCCGTGAA TGGAGAGTTC AGCCTGGACG ATCTCCAGCC GTGGCATTCT TTTGGGGCTG 1800

ACTCTGTGCC AGCCAACACA GAAAACGAAG TTGAGCCTGT TGATGCCCGC CCTGCTGCCG 1860
ACCGAGGACT GACCACTCGA CCAGGTTCTG GGTTGACAAA TATCAAGACG GAGGAGATCT 1920
CTGAAGTGAA GATGGATGCA GAATTCCGAC ATGACTCAGG ATATGAAGTT CATCATCAAA 1980
AATTGGTGTT CTTTGCAGAA GATGTGGGTT CAAACAAAGG TGCAATCATT GGACTCATGG 2040
TGGGCGGTGT TGTCATAGCG ACAGTGATCG TCATCACCTT GGTGATGCTG AAGAAGAAAC 2100
AGTACACATC CATTCATCAT GGTGTGGTGG AGGTTGACGC CGCTGTCACC CCAGAGGAGC 2160
GCCACCTGTC CAAGATGCAG CAGAACGGCT ACGAAAATCC AACCTAGAAG TTCTTTGAGC 2220
AGATGCAGAA CTAGACCCCC GCCACAGCAG CCTCTGAAGT TGGACAGCAA AACCATTGCT 2280
TCACTACCCA TCGGTGTCCA TTTATAGAAT AATGTGGGAA GAAACAAACC CGTTTTATGA 2340
TTTACTCATT ATCGCCTTTT GACAGCTGTG CTGTAACACA AGTAGATGCC TGAACTTGAA 2400
TTAATCCACA CATCAGTAAT GTATTCTATC TCTCTTTACA TTTTGGTCTC TATACTACAT 2460
TATTAATGGG TTTTGTGTAC TGTAAAGAAT TTAGCTGTAT CAAACTAGTG CATGAATAGA 2520
TTCTCTCCTG ATTATTTATC ACATAGCCCC TTAGCCAGTT GTATATTATT CTTGTGGTTT 2580
GTGACCCAAT TAAGTCCTAC TTTACATATG CTTTAAGAAT CGATGGGGGA TGCTTCATGT 2640
GAACGTGGGA GTTCAGCTGC TTCTCTTGCC TAAGTATTCC TTTCCTGATC ACTATGCATT 2700
TTAAAGTTAA ACATTTTTAA GTATTTCAGA TGCTTTAGAG AGATTTTTTT TCCATGACTG 2760
CATTTTACTG TACAGATTGC TGCTTCTGCT ATATTTGTGA TATAGGAATT AAGAGGATAC 2820
ACACGTTTGT TTCTTCGTGC CTGTTTTATG TGCACACATT AGGCATTGAG ACTTCAAGCT 2880
TTTCTTTTTT TGTCCACGTA TCTTTGGGTC TTTGATAAAG AAAAGAATCC CTGTTCATTG 2940
TAAGCACTTT TACGGGGCGG GTGGGGAGGG GTGCTCTGCT GGTCTTCAAT TACCAAGAAT 3000
TCTCCAAAAC AATTTTCTGC AGGATGATTG TACAGAATCA TTGCTTATGA CATGATCGCT 3060
TTCTACACTG TATTAGATAA ATAAATTAAA TAAAATAACC CCGGGCAAGA CTTTTCTTTG 3120
AAGGATGACT ACAGACATTA AATAATCGAA GTAATTTTGG GTGGGGAGAA GAGGCAGATT 3180
CAATTTTCTT TAACCAGTCT GAAGTTTCAT TTATGATACA AAAGAAGATG AAAATGGAAG 3240
TGGCAATATA AGGGGATGAG GAAGGCATGC CTGGACAAAC CCTTCTTTTA AGATGTGTCT 3300
TCAATTTGTA TAAAATGGTG TTTTCATGTA AATAAATACA TTCTTGGAGG AGC 3353

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5                   10                  15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
            20                  25                  30

Gly Leu Met Val Gly Gly Val Val Ile Ala
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
CAT CAA AAA TTG GTG TTC TTT                                        21
His Gln Lys Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
His Gln Lys Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
CAT CAA GAG TTG GTG TTC TTT                                     21
His Gln Glu Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
His Gln Glu Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
CAT CAA GTG TTG GTG TTC TTT                                             21
His Gln Val Leu Val Ph  Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
His Gln Val Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
CAT CAA GTG TTG GTG TTC TTT                                             21
His Gln Val Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
His Gln Val Leu Val Phe Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
CAT CAA AAA TTG GTG CCG TTT                                    21
His Gln Lys Leu Val Pro Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
His Gln Lys Leu Val Pro Phe
  1               5
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: lin ar

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GTC ATA GCG ACA GTG ATC GTC ATC ACC                          27
Val Ile Ala Thr Val Ile Val Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Val Ile Ala Thr Val Ile Val Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
GTC ATA GCG ACA GTG ATC ATC ATC ACC                                   27
Val Ile Ala Thr Val Ile Ile Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Val Ile Ala Thr Val Ile Ile Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
GTC ATA GCG ACA GTG ATC GGC ATC ACC                                   27
Val Ile Ala Thr Val Ile Gly Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Val Ile Ala Thr Val Ile Gly Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GTC ATA GCG ACA GTG ATC TTC ATC ACC                                  27
Val Ile Ala Thr Val Ile Phe Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Val Ile Ala Thr Val Ile Phe Ile Thr
  1               5
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
CAA AAA TTG GTG TTC TTT GCG GAA GAT                    27
Gln Lys Leu Val Phe Phe Ala Glu Asp
  1             5
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Gln Lys Leu Val Phe Phe Ala Glu Asp
  1             5
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
CAA AAA TTG GTG TTC TTT GCA CAA GAT                              27
Gln Lys Leu Val Phe Phe Ala Gln Asp
  1               5
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Gln Lys Leu Val Phe Phe Ala Gln Asp
  1               5
```

Claims

1. A nucleic acid molecule encoding an amyloid precursor mutein, wherein the nucleic acid molecule comprises, from the 5' end to the 3' end a nucleic acid sequence encoding a marker and a nucleic acid sequence encoding amino acid up to but excluding nucleic acids encoding BAP domain.

2. A nucleic acid molecule encoding an amyloid precursor mutein, wherein the nucleic acid molecule comprises, from the 5' end to the 3' end a nucleic acid sequence encoding BAP domain and a nucleic acid sequence encoding a marker.

3. A nucleic acid molecule which comprises the nucleic acid molecule of claim 1 ligated to the nucleic acid molecule of claim 2.

4. The nucleic acid molecule of claim 3, wherein the nucleic acid molecule is a nucleic acid molecule selected from the group consisting of DNA, cDNA or RNA.

5. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is selected from the group consisting of pCLL983, pCLL935, pCLL934 and pCLL913.

6. The nucleic acid molecule of claim 2, wherein the nucleic acid molecule is selected from the group consisting of pCLL947, pCLL914, pCLL937, pCLL949 and pCLL957.

7. The nucleic acid molecule of claim 3, wherein the nucleic acid molecule is selected from the group consisting of pCLL619, pCLL620, pCLL618, pCLL964, pCLL962, pCLL989, pCLL987, pCLL990, pCLL988, pCLL600, pCLL601, pCLL602, pCLL603, pCLL604, pCLL605, pCLL606 and pCLL607.

8. A vector comprising the nucleic acid molecule of claim 1, claim 2 or claim 3.

9. A cell comprising the nucleic acid molecule of claim 1, claim 2 or claim 3.

10. A recombinant polypeptide produced by the nucleic acid molecule of claim 1 , produced by the nucleic acid molecule of claim 2 or produced by the nucl ic acid molecule of claim 3.

11. A method of detecting the presence of the recombinant polypeptides of claim 10 in a sample, which comprises contacting an antibody directed to the marker and the sample under suitable conditions to favor the formation of an antibody-antigen complex, and detecting the presenc of any complex so formed.

Figure 1.

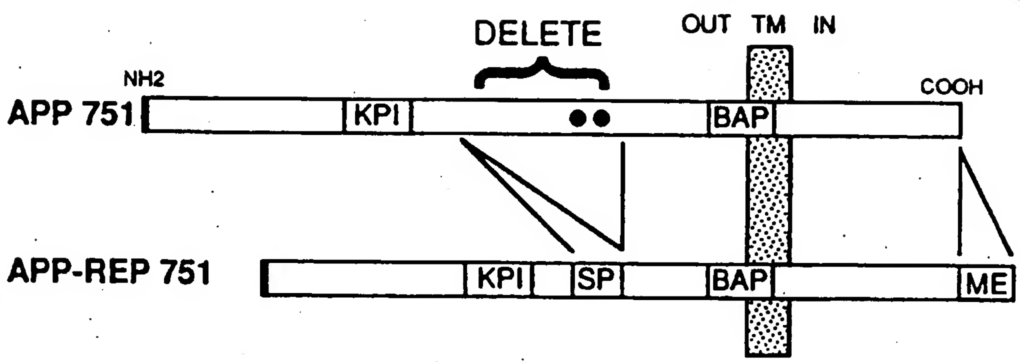

DELETE
OUT
TM
IN
NH2
COOH
APP 751
KPI
BAP
APP-REP 751
KPI
SP
BAP
ME

Figure 2.

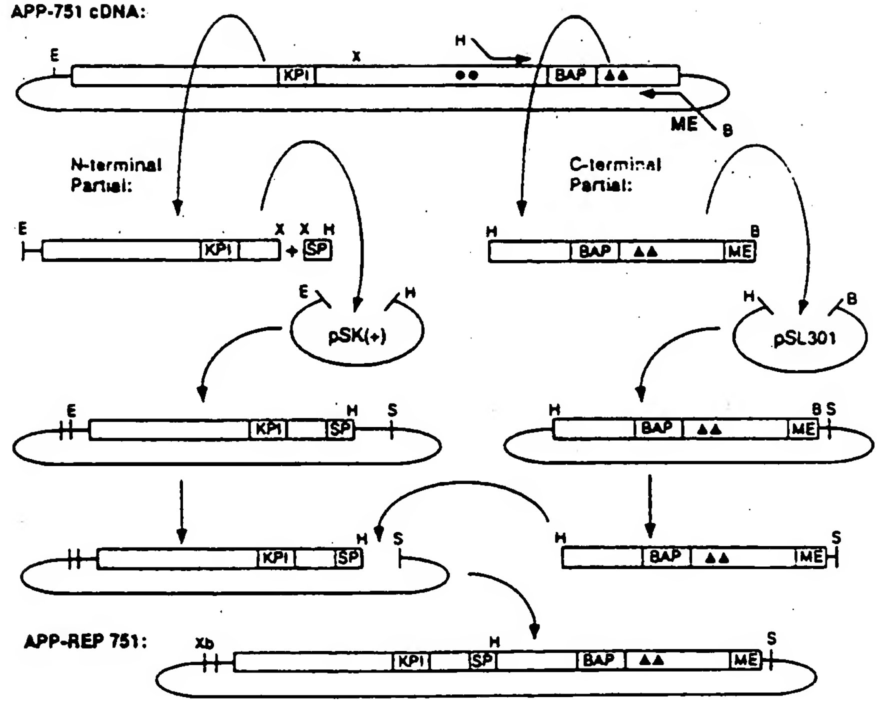

APP-751 cDNA:
E
X
H
KPI
BAP
ME
B
N-terminal Partial:
C-terminal Partial:
X X H
SP
pSK(+)
pSL301
APP-REP 751:
Xb
S

Figure 3.

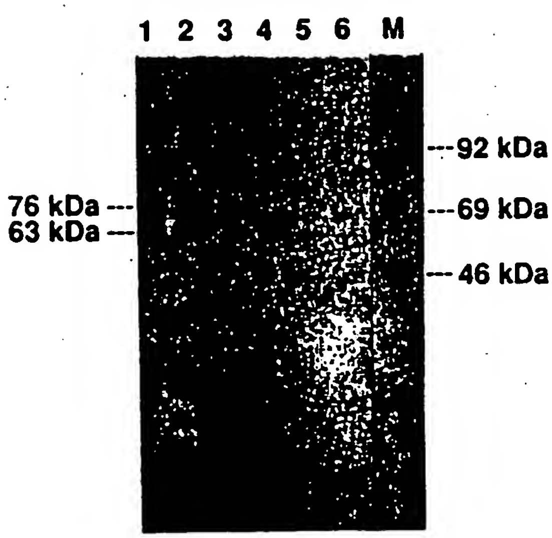
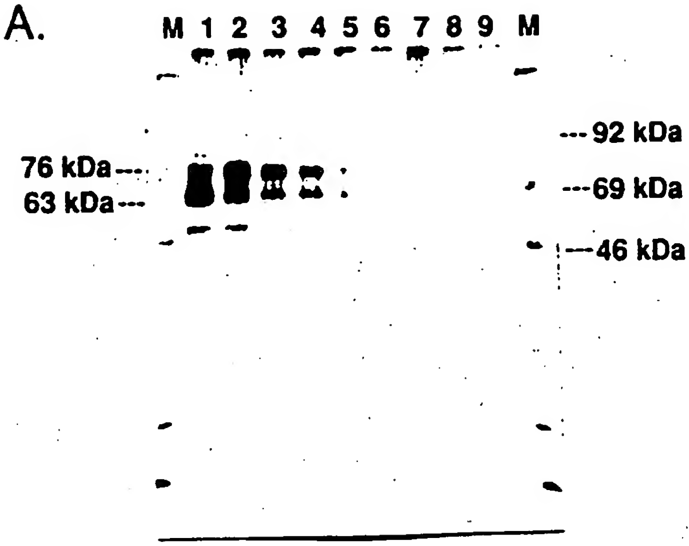


Figure 4.

A.


M 1 2 3 4 5 6 7 8 9 M
76 kDa---
63 kDa---
---92 kDa
---69 kDa
---46 kDa

B.


M 1 2 3 4 5 6 7 8 9 M
67 kDa---
---92 kDa
---69 kDa
--- 46 kDa

Figure 5.

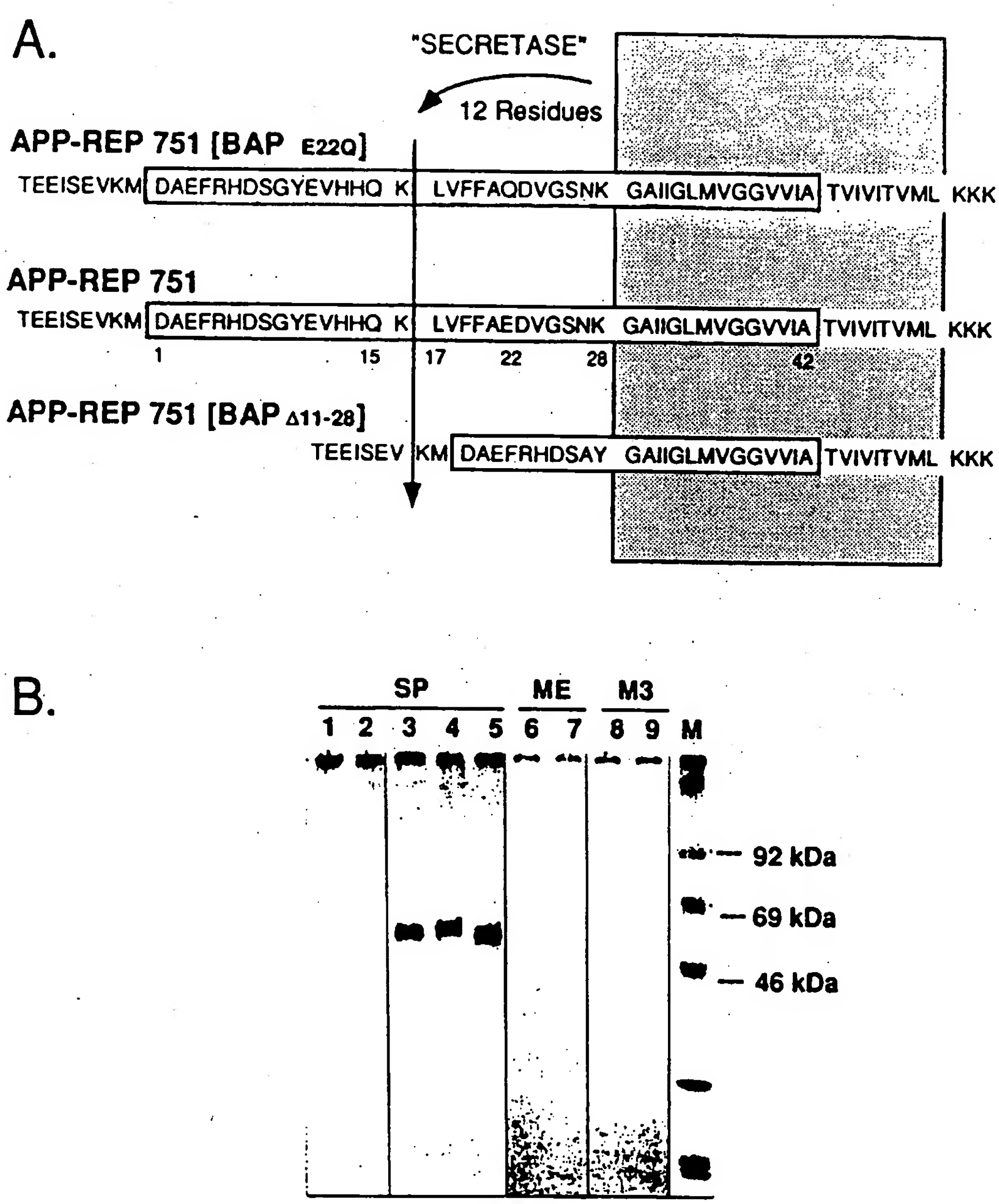
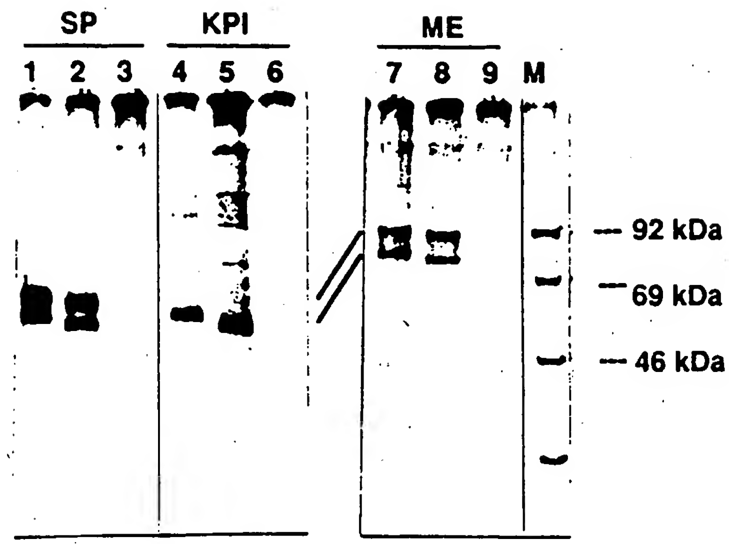

A.
"SECRETASE"
12 Residues
APP-REP 751 [BAP E22Q]
TEEISEVKM DAEFRHDSGYEVHHQ K LVFFAQDVGSNK GAIIGLMVGGVVIA TVIVITVML KKK
APP-REP 751
TEEISEVKM DAEFRHDSGYEVHHQ K LVFFAEDVGSNK GAIIGLMVGGVVIA TVIVITVML KKK
1 15 17 22 28 42
APP-REP 751 [BAP Δ11-28]
TEEISEV KM DAEFRHDSAY GAIIGLMVGGVVIA TVIVITVML KKK
B.
SP ME M3
1 2 3 4 5 6 7 8 9 M
92 kDa
69 kDa
46 kDa

Figure 5.

C.



D.

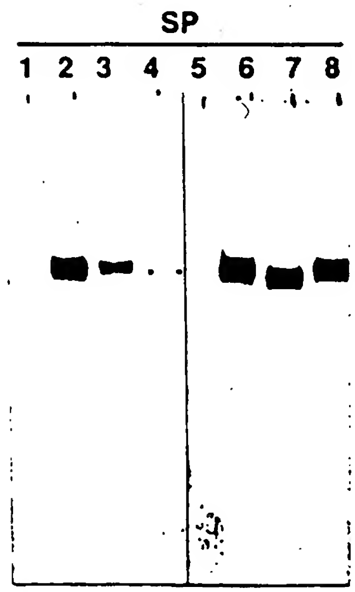


Figure 5.

E.

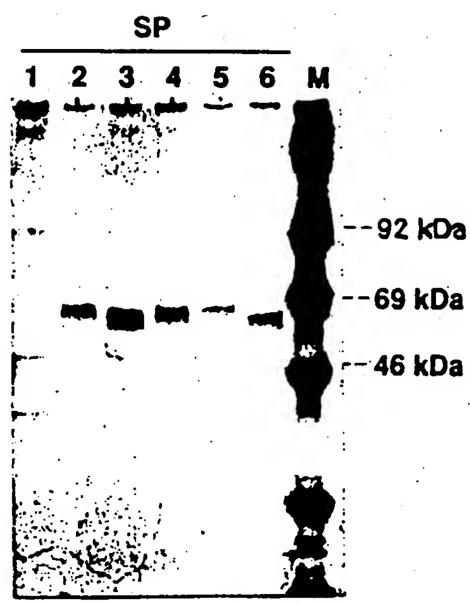


Figure 6.

A.

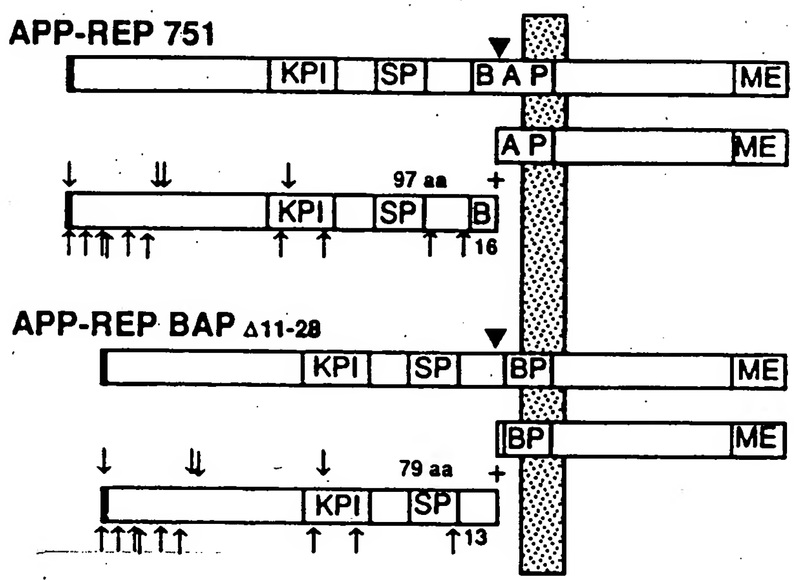
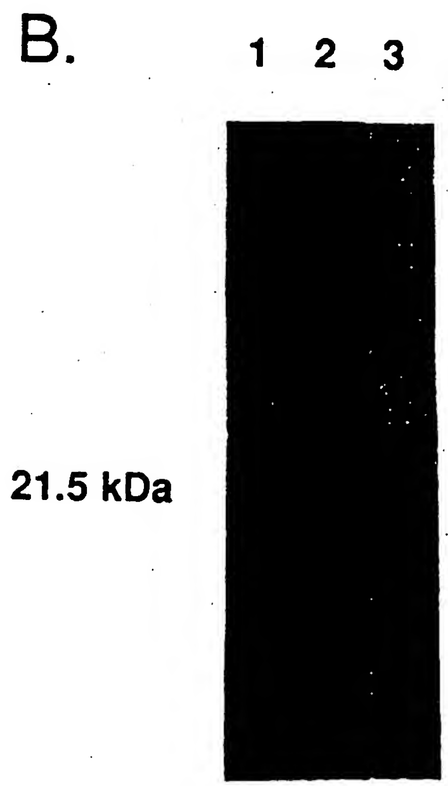

APP-REP 751
KPI
SP
B A P
ME
A P
ME
97 aa
KPI
SP
B
16
APP-REP BAP Δ11-28
KPI
SP
BP
ME
BP
ME
79 aa
KPI
SP
13

FIGURE 6.



```
SEQUENCE:  pCLL602  (APP-REP 751 protein)
VECTOR:    pcDNA-I-neo     (Invitrogen)
           pcDNA-I-neo-XS (JSJ modified polylinker to permit directional
              subcloning into XbaI-SalI sites)
INSERT:    XbaI-SalI fragment encoding APP-REP from pCLL964      16-1711
SEQUENCE:  5' polylinker:                                         2-47
              HindIII-XbaI from pcDNA-I-neo-XS                    2-15
              XbaI-EcoRI from pBluescript SK+                    16-47
           APP-REP 751:
              Amino-terminal partial from pCLL935):              48-1314
                 5' untranslated APP cDNA (from EcoRI)           48-195
                 N-terminal APP (to XhoI)                       196-1273
                 Substance P marker (XhoI to HindIII)          1274-1314
              Carboxy-terminal partial from pCLL947):          1314-1671
                 C-terminal APP and BAP (from novel HindIII)   1314-1656
                 Met-enkephalin marker (plus stop codon)       1657-1674
           3' polylinker:
              BamHI-SalI from pSL301                           1674-1711
              SalI-end of sequence from pcDNA-I-neo-XS         1712-1721
```

```
        10         20         30         40         50
         *          *          *          *          *
AAGCTTGGGG ATCCGCTCTA GAACTAGTGG ATCCCCCGGG CTGCAGGAAT
TTCGAACCCC TAGGCGAGAT CTTGATCACC TAGGGGGCCC GACGTCCTTA

        60         70         80         90        100
         *          *          *          *          *
TCGGGGGGGG CAGCGGTAGG CGAGAGCACG CGGAGGAGCG TGCGCGGGGC
AGCCCCCCCC GTCGCCATCC GCTCTCGTGC GCCTCCTCGC ACGCGCCCCG

       110        120        130        140        150
         *          *          *          *          *
CCCGGGAGAC GGCGGCGGTG GCGGCGCGGG CAGAGCAAGG ACGCGGCGGA
GGGCCCTCTG CCGCCGCCAC CGCCGCGCCC GTCTCGTTCC TGCGCCGCCT

       160        170        180        190
         *          *          *          *
TCCCACTCGC ACAGCAGCGC ACTCGGTGCC CCGCGCAGGG TCGCG
AGGGTGAGCG TGTCGTCGCG TGAGCCACGG GGCGCGTCCC AGCGC

   200          210           220          230          240
    *            *             *            *            *
ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG GCC GCC TGG ACG GCT
TAC GAC GGG CCA AAC CGT GAC GAG GAC GAC CGG CGG ACC TGC CGA
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala>

        250          260          270           280
         *            *            *             *
CGG GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT GGC CTG CTG GCT GAA
GCC CGC GAC CTC CAT GGG TGA CTA CCA TTA CGA CCG GAC GAC CGA CTT
Arg Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu>
```

FIGURE 7

```
290          300          310          320          330
 *            *            *            *            *
CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC ATG CAC ATG AAT GTC
GGG GTC TAA CGG TAC AAG ACA CCG TCT GAC TTG TAC GTG TAC TTA CAG
Pro Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val>

   340          350          360          370          380
    *            *            *            *            *
CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG ACC AAA ACC TGC ATT
GTC TTA CCC TTC ACC CTA AGT CTA GGT AGT CCC TGG TTT TGG ACG TAA
Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile>

      390          400          410          420          430
       *            *            *            *            *
GAT ACC AAG GAA GGC ATC CTG CAG TAT TGC CAA GAA GTC TAC CCT GAA
CTA TGG TTC CTT CCG TAG GAC GTC ATA ACG GTT CTT CAG ATG GGA CTT
Asp Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu>

         440          450          460          470          480
          *            *            *            *            *
CTG CAG ATC ACC AAT GTG GTA GAA GCC AAC CAA CCA GTG ACC ATC CAG
GAC GTC TAG TGG TTA CAC CAT CTT CGG TTG GTT GGT CAC TGG TAG GTC
Leu Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln>

            490          500          510          520
             *            *            *            *
AAC TGG TGC AAG CGG GGC CGC AAG CAG TGC AAG ACC CAT CCC CAC TTT
TTG ACC ACG TTC GCC CCG GCG TTC GTC ACG TTC TGG GTA GGG GTG AAA
Asn Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe>

530          540          550          560          570
 *            *            *            *            *
GTG ATT CCC TAC CGC TGC TTA GTT GGT GAG TTT GTA AGT GAT GCC CTT
CAC TAA GGG ATG GCG ACG AAT CAA CCA CTC AAA CAT TCA CTA CGG GAA
Val Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu>

   580          590          600          610          620
    *            *            *            *            *
CTC GTT CCT GAC AAG TGC AAA TTC TTA CAC CAG GAG AGG ATG GAT GTT
GAG CAA GGA CTG TTC ACG TTT AAG AAT GTG GTC CTC TCC TAC CTA CAA
Leu Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val>

      630          640          650          660          670
       *            *            *            *            *
TGC GAA ACT CAT CTT CAC TGG CAC ACC GTC GCC AAA GAG ACA TGC AGT
ACG CTT TGA GTA GAA GTG ACC GTG TGG CAG CGG TTT CTC TGT ACG TCA
Cys Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser>

         680          690          700          710          720
          *            *            *            *            *
GAG AAG AGT ACC AAC TTG CAT GAC TAC GGC ATG TTG CTG CCC TGC GGA
CTC TTC TCA TGG TTG AAC GTA CTG ATG CCG TAC AAC GAC GGG ACG CCT
Glu Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly>
```

FIGURE 7
(continued)

```
           730         740         750         760
             *           *           *           *
ATT GAC AAG TTC CGA GGG GTA GAG TTT GTG TGT TGC CCA CTG GCT GAA
TAA CTG TTC AAG GCT CCC CAT CTC AAA CAC ACA ACG GGT GAC CGA CTT
Ile Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu>

770         780         790         800         810
  *           *           *           *           *
GAA AGT GAC AAT GTG GAT TCT GCT GAT GCG GAG GAG GAT GAC TCG GAT
CTT TCA CTG TTA CAC CTA AGA CGA CTA CGC CTC CTC CTA CTG AGC CTA
Glu Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp>

    820         830         840         850         860
      *           *           *           *           *
GTC TGG TGG GGC GGA GCA GAC ACA GAC TAT GCA GAT GGG AGT GAA GAC
CAG ACC ACC CCG CCT CGT CTG TGT CTG ATA CGT CTA CCC TCA CTT CTG
Val Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp>

        870         880         890         900         910
          *           *           *           *           *
AAA GTA GTA GAA GTA GCA GAG GAG GAA GAA GTG GCT GAG GTG GAA GAA
TTT CAT CAT CTT CAT CGT CTC CTC CTT CTT CAC CGA CTC CAC CTT CTT
Lys Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu>

            920         930         940         950         960
              *           *           *           *           *
GAA GAA GCC GAT GAT GAC GAG GAC GAT GAG GAT GGT GAT GAG GTA GAG
CTT CTT CGG CTA CTA CTG CTC CTG CTA CTC CTA CCA CTA CTC CAT CTC
Glu Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu>

                970         980         990        1000
                  *           *           *           *
GAA GAG GCT GAG GAA CCC TAC GAA GAA GCC ACA GAG AGA ACC ACC AGC
CTT CTC CGA CTC CTT GGG ATG CTT CTT CGG TGT CTC TCT TGG TGG TCG
Glu Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser>

1010        1020        1030        1040        1050
   *           *           *           *           *
ATT GCC ACC ACC ACC ACC ACC ACC ACA GAG TCT GTG GAA GAG GTG GTT
TAA CGG TGG TGG TGG TGG TGG TGG TGT CTC AGA CAC CTT CTC CAC CAA
Ile Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val>

    1060        1070        1080        1090        1100
       *           *           *           *           *
CGA GAG GTG TGC TCT GAA CAA GCC GAG ACG GGG CCG TGC CGA GCA ATG
GCT CTC CAC ACG AGA CTT GTT CGG CTC TGC CCC GGC ACG GCT CGT TAC
Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met>

        1110        1120        1130        1140        1150
           *           *           *           *           *
ATC TCC CGC TGG TAC TTT GAT GTG ACT GAA GGG AAG TGT GCC CCA TTC
TAG AGG GCG ACC ATG AAA CTA CAC TGA CTT CCC TTC ACA CGG GGT AAG
Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe>
```

FIGURE 7
(continued)

```
      1160          1170           1180           1190           1200
         *             *              *              *              *
TTT TAC GGC GGA TGT GGC GGC AAC CGG AAC AAC TTT GAC ACA GAA GAG
AAA ATG CCG CCT ACA CCG CCG TTG GCC TTG TTG AAA CTG TGT CTT CTC
Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu>

         1210           1220           1230           1240
            *              *              *              *
TAC TGC ATG GCC GTG TGT GGC AGC GCC ATT CCT ACA ACA GCA GCC AGT
ATG ACG TAC CGG CAC ACA CCG TCG CGG TAA GGA TGT TGT CGT CGG TCA
Tyr Cys Met Ala Val Cys Gly Ser Ala Ile Pro Thr Thr Ala Ala Ser>

1250          1260           1270           1280           1290
   *             *              *              *              *
ACC CCT GAT GCC GTT GAC AAG TAT CTC GAG CGG CCC AAG CCC CAG CAG
TGG GGA CTA CGG CAA CTG TTC ATA GAG CTC GCC GGG TTC GGG GTC GTC
Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Arg Pro Lys Pro Gln Gln>

   1300          1310           1320           1330           1340
      *             *              *              *              *
TTC TTT GGC CTG ATG GGA AGC TTG ACA AAT ATC AAG ACG GAG GAG ATC
AAG AAA CCG GAC TAC CCT TCG AAC TGT TTA TAG TTC TGC CTC CTC TAG
Phe Phe Gly Leu Met Gly Ser Leu Thr Asn Ile Lys Thr Glu Glu Ile>

     1350          1360           1370           1380           1390
        *             *              *              *              *
TCT GAA GTG AAG ATG GAT GCA GAA TTC CGA CAT GAC TCA GGA TAT GAA
AGA CTT CAC TTC TAC CTA CGT CTT AAG GCT GTA CTG AGT CCT ATA CTT
Ser Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu>

        1400          1410           1420           1430           1440
           *             *              *              *              *
GTT CAT CAT CAA AAA TTG GTG TTC TTT GCA GAA GAT GTG GGT TCA AAC
CAA GTA GTA GTT TTT AAC CAC AAG AAA CGT CTT CTA CAC CCA AGT TTG
Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn>

           1450          1460           1470           1480
              *             *              *              *
AAA GGT GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC ATA GCG ACA
TTT CCA CGT TAG TAA CCT GAG TAC CAC CCG CCA CAA CAG TAT CGC TGT
Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr>

1490          1500           1510           1520           1530
   *             *              *              *              *
GTG ATC GTC ATC ACC TTG GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC
CAC TAG CAG TAG TGG AAC CAC TAC GAC TTC TTC TTT GTC ATG TGT AGG
Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser>

   1540          1550           1560           1570           1580
      *             *              *              *              *
ATT CAT CAT GGT GTG GTG GAG GTT GAC GCC GCT GTC ACC CCA GAG GAG
TAA GTA GTA CCA CAC CAC CTC CAA CTG CGG CGA CAG TGG GGT CTC CTC
Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu>
```

FIGURE 7
(c ntinued)

```
   )0          1600          1610          1620           1630
   *             *             *             *              *
CGC CAC CTG TCC AAG ATG CAG CAG AAC GGC TAC GAA AAT CCA ACC TAC
GCG GTG GAC AGG TTC TAC GTC GTC TTG CCG ATG CTT TTA GGT TGG ATG
Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr>

      1640          1650          1660          1670         1680
         *             *             *             *            *
AAG TTC TTT GAG CAG ATG CAG AAC TAT GGG GGC TTC ATG TAG GATCCA
TTC AAG AAA CTC GTC TAC GTC TTG ATA CCC CCG AAG TAC ATC CTAGGT
Lys Phe Phe Glu Gln Met Gln Asn Tyr Gly Gly Phe Met ***

      1690       1700       1710         1720
         *          *          *            *
TATATAGGGC CCGGGTTAT AATTACCTCA GGTCGACCTA GA
ATATATCCCG GGCCCAATA TTAATGGAGT CCAGCTGGAT CT
```

FIGURE 7
(continued)

```
Total of 12 files.
PRMRD3>TYPE APP770.
LOCUS       HUMAFPA4      3353 bp ss-mRNA            PRI       15-JUN-1989
Human amyloid A4 mRNA, complete cds.
ACCESSION   Y00264
KEYWORDS    amyloid fibril protein; cell surface glycoprotein.
SOURCE      human (Homo sapiens).
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Metazoa; Chordata; Vertebrata; Mammalia;
            Theria; Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 3353; enum. -146 to 3207, no zero)
  AUTHORS   Kang,J., Lemaire,H.G., Unterbeck,A., Salbaum,J.M., Masters,C.L.,
            Grzeschik,K.-H., Multhaup,G., Beyreuther,K. and Mueller-Hill,B.
  TITLE     The precursor of Alzheimer's disease amyloid A4 protein resembles a
            cell-surface receptor
  JOURNAL   Nature 325, 733-736 (1987)
  STANDARD  simple automatic
REFERENCE   2  (bases 1 to 3353; enum. 1 to 3353)
  AUTHORS   Mueller Hill,B.
  JOURNAL   Unpublished (1987) Submitted to the EMBL data library.
  STANDARD  simple automatic
COMMENT     *source: tissue=cortex of brain; *source: developmental
            stage=5-month-old aborted fetus;
              EMBL features not translated to GenBank features:
              key         from     to        description
              SITE        3080    3085       polyA signal
              SITE        3089    3094       polyA signal
              SITE        3331    3336       polyA signal
              POLYA       3353    3353       polyA site
FEATURES      from  to/span      description
     pept      147     2234      amyloid A4 /nomgen="APP" /map="21q21.2"
                                 /hgml_locus_uid="LG0136J"
BASE COUNT      922 a      745 c     867 g     819 t
ORIGIN

      App770.  Length: 3353  May 29, 1990  11:30  Check: 6510  ..

        1  AGTTTCCTCG GCAGCGGTAG GCGAGAGCAC GCGGAGGAGC GTGCGCGGGG

       51  CCCCGGGAGA CGGCGGCGGT GGCGGCGCGG GCAGAGCAAG GACGCGGCGG

      101  ATCCCACTCG CACAGCAGCG CACTCGGTGC CCCGCGCAGG GTCGCGATGC

      151  TGCCCGGTTT GGCACTGCTC CTGCTGGCCG CCTGGACGGC TCGGGCGCTG

      201  GAGGTACCCA CTGATGGTAA TGCTGGCCTG CTGGCTGAAC CCCAGATTGC

      251  CATGTTCTGT GGCAGACTGA ACATGCACAT GAATGTCCAG AATGGGAAGT

      301  GGGATTCAGA TCCATCAGGG ACCAAAACCT GCATTGATAC CAAGGAAGGC

      351  ATCCTGCAGT ATTGCCAAGA AGTCTACCCT GAACTGCAGA TCACCAATGT

      401  GGTAGAAGCC AACCAACCAG TGACCATCCA GAACTGGTGC AAGCGGGGCC

      451  GCAAGCAGTG CAAGACCCAT CCCCACTTTG TGATTCCCTA CCGCTGCTTA

      501  GTTGGTGAGT TTGTAAGTGA TGCCCTTCTC GTTCCTGACA AGTGCAAATT

      551  CTTACACCAG GAGAGGATGG ATGTTTGCGA AACTCATCTT CACTGGCACA

      601  CCGTCGCCAA AGAGACATGC AGTGAGAAGA GTACCAACTT GCATGACTAC

      651  GGCATGTTGC TGCCCTGCGG AATTGACAAG TTCCGAGGGG TAGAGTTTGT

      701  GTGTTGCCCA CTGGCTGAAG AAAGTGACAA TGTGGATTCT GCTGATGCGG
```

FIGURE 8

751 AGGAGGATGA CTCGGA[illegible]GTC TGGTGGGGCG GAGCAGACAC AG[illegible]ATGCA
801 GATGGGAGTG AAGACAAAGT AGTAGAAGTA GCAGAGGAGG AAGAAGTGGC
851 TGAGGTGGAA GAAGAAGAAG CCGATGATGA CGAGGACGAT GAGGATGGTG
901 ATGAGGTAGA GGAAGAGGCT GAGGAACCCT ACGAAGAAGC CACAGAGAGA
951 ACCACCAGCA TTGCCACCAC CACCACCACC ACCACAGAGT CTGTGGAAGA
1001 GGTGGTTCGA GTTCCTACAA CAGCAGCCAG TACCCCTGAT GCCGTTGACA
1051 AGTATCTCGA GACACCTGGG GATGAGAATG AACATGCCCA TTTCCAGAAA
1101 GCCAAAGAGA GGCTTGAGGC CAAGCACCGA GAGAGAATGT CCCAGGTCAT
1151 GAGAGAATGG GAAGAGGCAG AACGTCAAGC AAAGAACTTG CCTAAAGCTG
1201 ATAAGAAGGC AGTTATCCAG CATTTCCAGG AGAAAGTGGA ATCTTTGGAA
1251 CAGGAAGCAG CCAACGAGAG ACAGCAGCTG GTGGAGACAC ACATGGCCAG
1301 AGTGGAAGCC ATGCTCAATG ACCGCCGCCG CCTGGCCCTG GAGAACTACA
1351 TCACCGCTCT GCAGGCTGTT CCTCCTCGGC CTCGTCACGT GTTCAATATG
1401 CTAAAGAAGT ATGTCCGCGC AGAACAGAAG GACAGACAGC ACACCCTAAA
1451 GCATTTCGAG CATGTGCGCA TGGTGGATCC CAAGAAAGCC GCTCAGATCC
1501 GGTCCCAGGT TATGACACAC CTCCGTGTGA TTTATGAGCG CATGAATCAG
1551 TCTCTCTCCC TGCTCTACAA CGTGCCTGCA GTGGCCGAGG AGATTCAGGA
1601 TGAAGTTGAT GAGCTGCTTC AGAAAGAGCA AAACTATTCA GATGACGTCT
1651 TGGCCAACAT GATTAGTGAA CCAAGGATCA GTTACGGAAA CGATGCTCTC
1701 ATGCCATCTT TGACCGAAAC GAAAACCACC GTGGAGCTCC TTCCCGTGAA
1751 TGGAGAGTTC AGCCTGGACG ATCTCCAGCC GTGGCATTCT TTTGGGGCTG
1801 ACTCTGTGCC AGCCAACACA GAAAACGAAG TTGAGCCTGT TGATGCCCGC
1851 CCTGCTGCCG ACCGAGGACT GACCACTCGA CCAGGTTCTG GGTTGACAAA
1901 TATCAAGACG GAGGAGATCT CTGAAGTGAA GATGGATGCA GAATTCCGAC
1951 ATGACTCAGG ATATGAAGTT CATCATCAAA AATTGGTGTT CTTTGCAGAA
2001 GATGTGGGTT CAAACAAAGG TGCAATCATT GGACTCATGG TGGGCGGTGT
2051 TGTCATAGCG ACAGTGATCG TCATCACCTT GGTGATGCTG AAGAAGAAAC
2101 AGTACACATC CATTCATCAT GGTGTGGTGG AGGTTGACGC CGCTGTCACC
2151 CCAGAGGAGC GCCACCTGTC CAAGATGCAG CAGAACGGCT ACGAAAATCC
2201 AACCTACAAG TTCTTTGAGC AGATGCAGAA CT

AGACCCCC GCCACAGCAG
2251 CCTCTGAAGT TGGACAGCAA AACCATTGCT TCACTACCCA TCGGTGTCCA

FIGURE 8
(c ntinued)

2301 TTTATAGAAT AATGTGCCAA GAAACAAACC CGTTTTATGA TTTACTCATT
2351 ATCGCCTTTT GACAGCTGTG CTGTAACACA AGTAGATGCC TGAACTTGAA
2401 TTAATCCACA CATCAGTAAT GTATTCTATC TCTCTTTACA TTTTGGTCTC
2451 TATACTACAT TATTAATGGG TTTTGTGTAC TGTAAAGAAT TTAGCTGTAT
2501 CAAACTAGTG CATGAATAGA TTCTCTCCTG ATTATTTATC ACATAGCCCC
2551 TTAGCCAGTT GTATATTATT CTTGTGGTTT GTGACCCAAT TAAGTCCTAC
2601 TTTACATATG CTTTAAGAAT CGATGGGGGA TGCTTCATGT GAACGTGGGA
2651 GTTCAGCTGC TTCTCTTGCC TAAGTATTCC TTTCCTGATC ACTATGCATT
2701 TTAAAGTTAA ACATTTTTAA GTATTTCAGA TGCTTTAGAG AGATTTTTTT
2751 TCCATGACTG CATTTTACTG TACAGATTGC TGCTTCTGCT ATATTTGTGA
2801 TATAGGAATT AAGAGGATAC ACACGTTTGT TTCTTCGTGC CTGTTTTATG
2851 TGCACACATT AGGCATTGAG ACTTCAAGCT TTTCTTTTTT TGTCCACGTA
2901 TCTTTGGGTC TTTGATAAAG AAAAGAATCC CTGTTCATTG TAAGCACTTT
2951 TACGGGGCGG GTGGGGAGGG GTGCTCTGCT GGTCTTCAAT TACCAAGAAT
3001 TCTCCAAAAC AATTTTCTGC AGGATGATTG TACAGAATCA TTGCTTATGA
3051 CATGATCGCT TTCTACACTG TATTACATAA ATAAATTAAA TAAAATAACC
3101 CCGGGCAAGA CTTTTCTTTG AAGGATGACT ACAGACATTA AATAATCGAA
3151 GTAATTTTGG GTGGGGAGAA GAGGCAGATT CAATTTTCTT TAACCAGTCT
3201 GAAGTTTCAT TTATGATACA AAAGAAGATG AAAATGGAAG TGGCAATATA
3251 AGGGGATGAG GAAGGCATGC CTGGACAAAC CCTTCTTTTA AGATGTGTCT
3301 TCAATTTGTA TAAAATGGTG TTTTCATGTA AATAAATACA TTCTTGGAGG
3351 AGC

PRMRD3>

FIGURE 8
(continued)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 93 10 5718

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 451 700 (MILES INC.) *Examples 6-8 and 12; Table II; Claims* ---	1-4,8-11	C12N15/12 C12N15/62 C07K15/00 C12N5/10
X	WO-A-9 014 840 (CALIFORNIA BIOTECHNOLOGY INC.) *page 43, line 20 - page 45, line 34; Example 8; Claims* ---	1,4,8-10	
X	WO-A-8 803 951 (CALIFORNIA BIOTECHNOLOGY INC.) *page 41, line 1 - page 43, line 22; page 52, line 1-23; claims* ---	1,4,8-10	
A	WO-A-9 001 540 (CALIFORNIA BIOTECHNOLOGY INC.) *Example II; Claims* ---	1	
D,A	SCIENCE vol. 248, 1990, pages 492 - 495 S.S. SISODIA ET AL.; 'Evidence that beta-amyloid protein in Alzheimer's Disease is not derived by normal processing' *whole document* ---	1	**TECHNICAL FIELDS SEARCHED (Int. Cl.5)** C12N C07K
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 267, 1992, pages 25602 - 25608 S.R. SAHASRABUDHE ET AL.; 'Release of amino-terminal fragments from amyloid precursor protein reporter and mutated derivatives in cultured cells' *whole document* -----	1-11	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	16 AUGUST 1993	YEATS S.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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L : document cited for other reasons

& : member of the same patent family, corresponding document